EXTRACTION, PHYTOCHEMICAL INVESTIGATION AND BIOLOGICAL EVALUATION OF BARK OF *SYZYGIUM CUMINI* (L.).

Dissertation submitted to

தமிழ்நாடு டாக்டர் எம்.ஜி.ஆர். மருத்துவப் பல்கலைக்கழகம்

The Tamil Nadu Dr.M.G.R. Medical University

In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL CHEMISTRY

By

(Reg No: 261515351)

Under the Guidance and Supervision of

Prof. Dr. N. VENKATESHAN., M. pharm. Ph. D.,
Department of Pharmaceutical Chemistry

ARULMIGU KALASALINGAM COLLEGE OF PHARMACY
KRISHNANKOIL – 626 126.

MAY- 2017

CERTIFICATE

This is to certify that the thesis entitled "**EXTRACTION, PHYTOCHEMICAL INVESTIGATION AND BIOLOGICAL EVALUATION OF BARK OF *SYZYGIUM CUMINI (L.)***" submitted by **Reg. No 261515351** was carried out in the Department of Pharmaceutical Chemistry Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil – 626126, which is affiliated to The Tamil Nadu Dr. M. G. R Medical University, Chennai, under the Direct Supervision and Guidance for the Partial fulfillment of Degree of Master of Pharmacy in Pharmaceutical Chemistry.

Place **:**Krishnankoil

Date **:**

Prof. Dr. N. VENKATESHAN

Professor & Principal

Arulmigu Kalasalingam College of Pharmacy

Anand Nagar, Krishinankoil – 626 126.

EVALUATION CERTIFICATE

This to certify that the dissertation work entitled "**EXTRACTION, PHYTOCHEMICAL INVESTIGATION AND BIOLOGICAL EVALUATION OF BARK OF *SYZYGIUM CUMINI (L.)***" submitted by **Reg. No 261515351** to The Tamil Nadu Dr. M. G. R Medical University, Chennai, in Partial fulfillment of the requirement for the award of the Degree of Master of Pharmacy in Pharmaceutical Chemistry is evaluated by,

Date :

Center: Arulmigu Kalasalingam College of Pharmacy,
Anand Nagar,
Krishnankoil – 626 126.

Examiners:

1.

2.

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J ARUNPANDIYAN

DEDICATED TO

LORD

AND

MY PARENTS

Dr. D. Stephen
Professor
Department of Botany

The American College
Madurai-2
Mobile no: 9944792299

Authentification Certificate

This is to certify that the plant specimen brought to me by J.ARUNPANDIYAN (Reg.No:261515351), Final year M.Pharm (2016-2017), **Department of Pharmaceutical chemistry**, **Arulmigu Kalasalingam College Of Pharmacy,** Krishnankoil, Srivilliputhur, has been identified as ***Syzygium cumini*** belonging to the family **Myrtaceae** .

Date: 10.8.2016.

Dr. D. Stephen

Dr. D. STEPHEN, Ph.D.,
ASST. PROFESSOR IN BOTANY
THE AMERICAN COLLEGE
MADURAI - 625 002
TAMILNADU-INDIA

LIST OF ABBREVIATIONS

1.	NaOH	-	Sodium hydroxide
2.	$Fecl_3$	-	Ferric chloride
3.	DPPH	-	2,2-diphenyl-1- picryl hydrazyl
4.	H_2O_2	-	Hydrogen peroxide
5.	$CHCl_3$	-	Chloroform
6.	Pet.ether	-	Petroleum ether
7.	Std	-	Standard
8.	Mm	-	Millimetre
9.	µg	-	Microgram
10.	ML	-	Milli liter
11.	Car	-	Carbohydrate
12.	Pro	-	Protein
13.	AA	-	Aminoacid
14.	Ster	-	Steroid
15.	Alk	-	Alkaloid
16.	Tan	-	Tannins
17.	Fla	-	Flavanoid
18.	F&O	-	Fat &Oils
19.	Vol	-	Volatile oils
20.	*E.coli*	-	*Escherichia coli.*
21.	*P.aeruginosa*	-	*Pseudomonas aeruginosa*
22.	*S.aureus*	-	*Staphylococcus aureus*
23.	*B.substilus*	-	*Bacillus subtilis*
24.	*S.cumini*	-	*Syzygium cumini*

INDEX

INTRODUCTION

INTRODUCTION

Since disease, decay and death have always co-existed with life, the study of diseases and their treatment must also have been contemporaneous with the down of the human intellect. It is greatly to the credit of the people of India that they were acquainted with a far larger number of medicinal plants than the natives of any other country on the face of the earth.

About a generation ago, the use of plants and herbs as remedial agents was greatly discredited. In the same way as we divide the civilization into 4 stages, we may recognize 4 stages in the development of the implements in the treatment of disease. In the first stage crude drugs were employed, prepared in the roughest manner such as powder form. In the next stage, these were converted into more active and more manageable forms, such as extracts or solutions. In the third stage pure active principles, separated from the crude drugs were employed. In the 4th stage instead of attempting to extract our medicines from the natural products, we seek to make for ourselves such substances as shall possess the particular action we desire. From this it can be said that the study of medicinal plants serve as a basis of synthetic chemistry also.

The study of medicinal plants is neglected by medical men all over the world, but more so in India. It is our misfortune that the chemistry and pharmacology of most of these plants have not been properly investigated. The ease and cheapness with which these are procurable the marvelous powers that are attributed to them in the cure of different diseases encourages us to investigate their properties and prove their pharmacological actions.

The *Syzygium cumini* (L.) Skeels (*Syns. Syzygium jambolana* DC, *Eugenia cumini* Druce, *Eugenia jambolana* Lam.),commonly known as Jamun, belongs to the family Myrtaceae or *Myrtle*[1]. The other common names of jamun are Indian blackberry, Java plum, Jambu, black plum and Jambul *etc*[2]*. S. cumini* is an emerging fruit crop of the twenty-first century. The jamun fruit have high medicinal value as well as different plant parts possess varied uses to mankind.In the production of jamun, India get second place in all over the world. Uttar Pradesh state is the largest producer followed by Maharashtra, Tamil Nadu, Gujarat, Assam. Ripe fruits are purpulish with high content of anthocyanin with a pleasant odour,astringent taste and

are processed to make vinegar, jam, jellies and squash. It gives authority of due to the presence of the various phytochemical constituents such as alkaloids, fatty acids, steroids and tannins. It is multipurpose tree cultivating for varied uses as a road bordered by trees for wind break, preserving fishing nets and tanning leather by brown dye which obtained from bark due to its have high value of tannin content. The wood also used for building, carpentry, fuel wood and preparation of different agricultural implements.

Religious Veneration

In Hindu religious, Jamun belived as 'fruit of the gods' especially in Gujarat state. In southern Asia, the tree is worship with reverence by Buddhists, and it is regared holy to Krishna. The leaves and fruits are faithful as Ganesha or Vinayaka[3-4].

Taxonomy[5]

The genus name *Syzygium* is derived from the Greek word *Syzygos,* meaning yoked together, possibly referring to the paired leaves. The taxonomy of the *S. cumini* (L.). is as follows-

Kingdom	: Plantae - Plants
Subkingdom	: Tracheobionta - Vascular plants
Super division	: Spermatophyta - Seed plants
Division	: Magnoliophyta - Flowering plants
Class	: Magnoliopsida - Dicotyledons
Subclass	: Rosidae
Order	: Myrtales
Family	: Myrtaceae - Myrtle family
Genus	: *Syzygium* P. Br. ex Gaertn.
Complete scientific name	: *Syzygium cumini* (L.) Skeels

Botanical Description[5]

Evergreen to 25 m tall, with young stems grayish white and lower bark discolored. Leaves are opposite, simple, oblong,glossy 5-10 cm long,tips are very short point, petiols to 3 cm long; Leaf midrip prominent, yellowish. Flowers white to pinkish about 1 cm across,4 petals,fused into a cap;many stamens.Fruit an ovoid,1-seeded berry to 2 cm long,dark purplish red, shiny ,with white to lavender fles

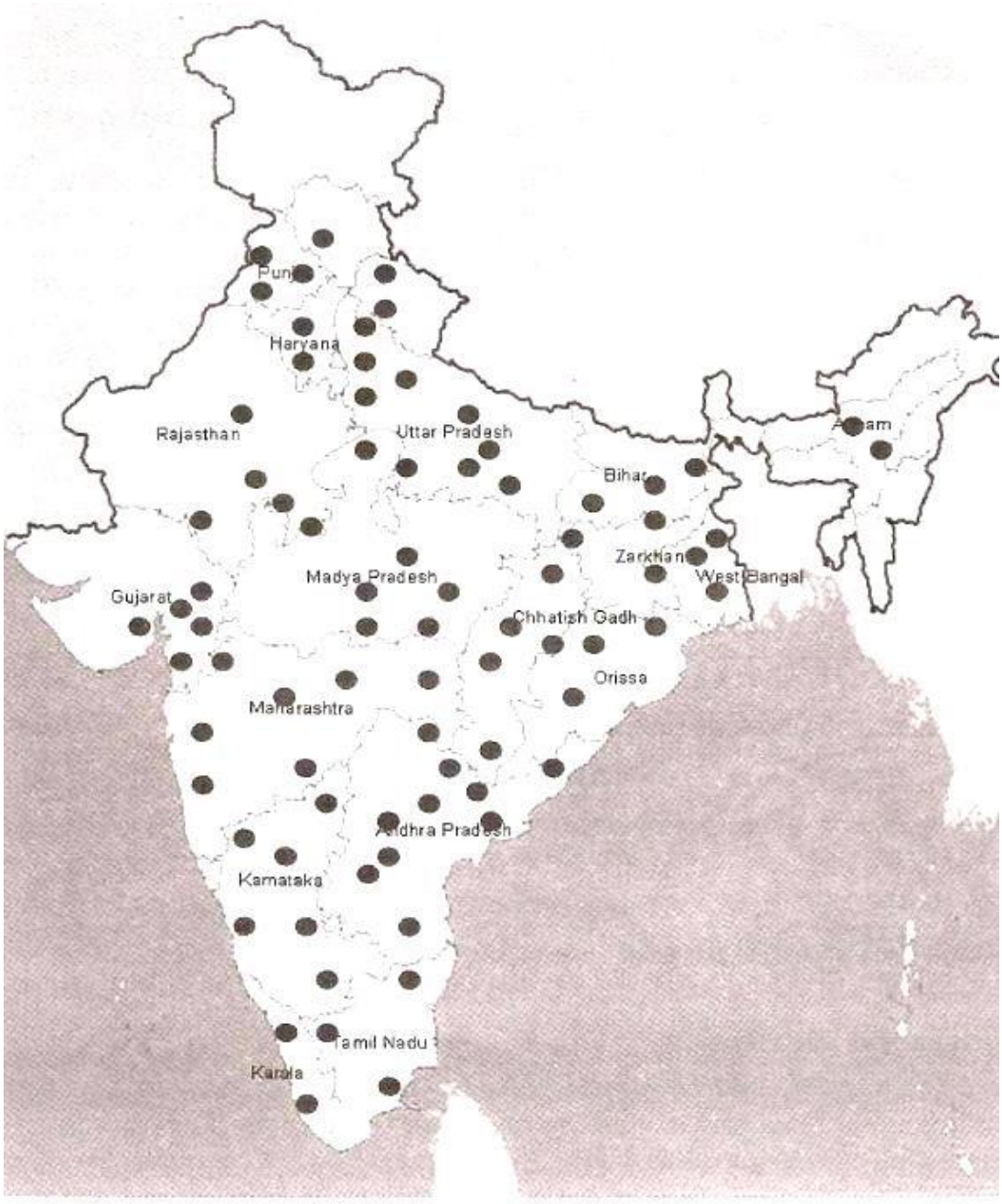
LEAVES

FRUITS

FLOWER,LEAVES, FRUITS

BARK

Distribution of *Syzygium* species in India[6]



Pharmacological potential of different parts of *S. cumini* (L.).

The *S. cumini* (L.). occupy considerable place in our life that have efficacy to upgrade the unfavourable effects of many diseases. Its reference had been quoted in Rigveda and Atharva Veda. Since the time of Charaka and Susruta, it is also used extensively in the several traditional systems of medicine resembling in the Ayurveda, Unani, Siddha, Homeopathy, alternative and complementary medicine[4]. The jamun plant was used either alone or in combination with other hypoglycemic plants in the treatment of diabetes before the discovery of insulin. Its bark is acrid which is used in the treatment for sore throat, bronchitis, asthma, biliousness, dysentery, blood impurities and to cure ulcers[2]. The decocsation of bark is used as lotion for removing ringworm of the head [4]and also significantly decrease in blood glucose levels in mice [7].β-sitoterol present in the unsaponifiable matter of seed fat which is used in the treatment for antidiabetic, anti inflammatory, hepatoprotective, anti-hyperlipidemic, diuretic and antibacterial activities have been reported in various extracts of *Syzygium cumini*(L.).seeds[8] . The different parts of jamun tree were make uses for cure of various diseases in the formation of powder, decoction, juice or paste[9]. Fresh leaf juice is taken orally for stomach pain[10]. In the Siddha system of medicine, the teeth and the gums are strengthening by the ash of the leaves. *S. cumini* is considered to be a haematinic, semen promoting and to decrease excessive heat of the body[4]. Several experimental and clinical studies have been confirmed antidiabetic activities of various parts of jamun[11-15]. The various extracts of different parts of jamun possess a range of pharmacological properties such as antibacterial[16-19], antimicrobial[20], antifungal[21], antiviral[22], antioxidant and free radical scavenging activity[23-27],cardioprotective[28],anti-inflammatory[29-30], neuropsychopharmacological[31], antiallergic[32], radioprotective[33], chemopreventive[34], larvicidal[35], gastroprotective and antiulcerogenic[36] activities.

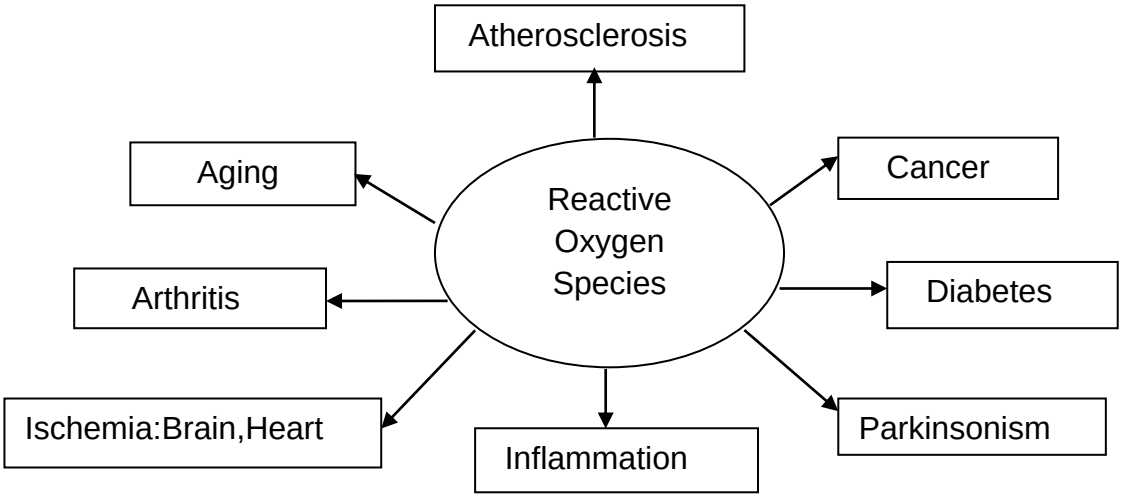
ANTIOXIDANT

Human society is going under serious predicament. We have workload and social/professional pressure by the reason of better lifestyle and more comfortable life leading Organic substances which are not stable under atmosphere conditions. Number of factors especially by the action of reactive oxygen-derived species degraded all organic substances like oils, food products, plastics, rubber products etc. which process known as auto oxidation, with loss of chemical and physical

properties. Food and oxygen which are the basic need of human life are actually involved in complicated incompatible interactions

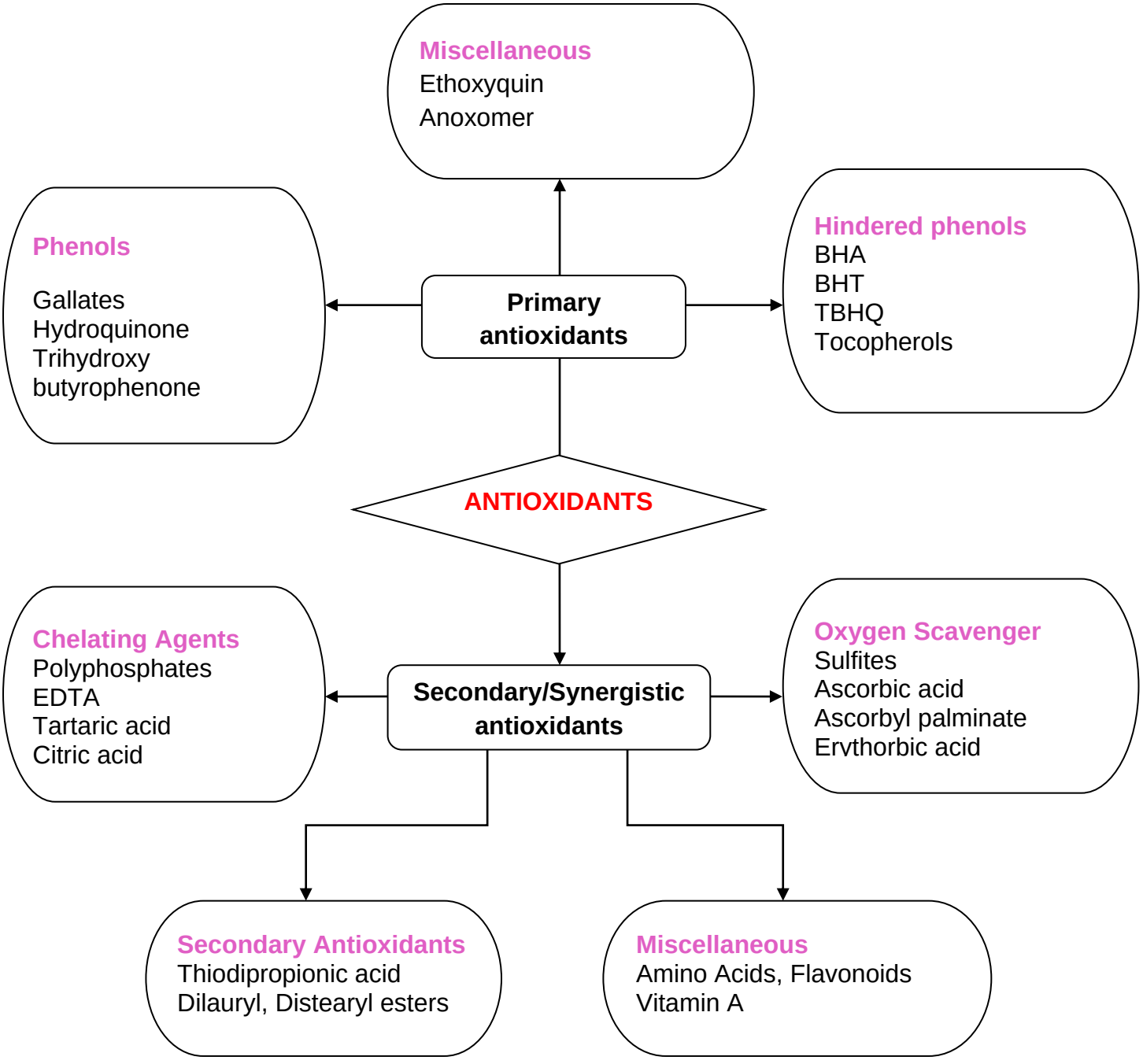
Oxidative stress in health and diseases

Free radical is defined as any atom (e.g. oxygen, nitrogen) have at least one unpaired electron in the outermost shell, and is accomplished of independent subsistence. Oxygen is the most significant element for life which is the major resource of free radicals. Oxygen is use by cell for generate energy, which leads to created free radicals are as a end result of ATP (adenosine triphosphate) production by the mitochondria. The free radicals participate a twin role as both toxic and beneficial compounds Due to formation of free radicals low or moderate levels contribute to good physical functions on cellular responses and immune function in human health and development when they are not too much. Free radicals occurs not only normal cellular process always occurs upon revelation to certain chemicals such as polycyclic aromatic hydrocarbon, cadmium, lead, etc., radiation, cigarette smoke, and high fat diet. A balance between formations of is essential for normal cellular function. When balance of free radicals formation and their detoxification unequal which causes production of damaging species or antioxidants present in low levels which leads to oxidative stress in cell and end result of cell damaged. If the cell damage causes genetic instability that which produce chronic diseases including cancer[37]. The ROS can cause disturb to biological systems which end of results can produce degenerative diseases like mutagenesis, carcinogenesis, circulatory disturbance, and aging[38-44]



The excess of free radicals counterbalance by the antioxidants which leads to shield the cells against their toxic effects and end of the results contribute to disease prevention. Antioxidants are almost worldwide in normally consumed herbal food products. When we taken moderate concentration of antioxidant as long as they have several positive health effects. ROS are neutralized by antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx)

CLASSIFICATION OF ANTIOXIDANTS



Primary antioxidants

Primary antioxidants defiened as the free radical chain reaction terminate by donating hydrogen or electrons which results to converting to more stable products. They are further classified in to phenolic, hindered phenolic group. Primary antioxidants react with free radicals especially peroxy free radicals by donating their active hydrogen which leads to inhibit the propagation step.

The peroxy antioxidant compounds forming by the antioxidant free radical interfere with chain- propagation reactions.

$$AH + ROO^{\bullet} \longrightarrow A^{\bullet} + ROOH$$

$$A^{\bullet} + ROO^{\bullet} \longrightarrow ROOA$$

AH = Hydrogen donating antioxidant $ROO^{\bullet}$ = Peroxy free radical

$A^{\bullet}$ = Antioxidant free radical ROOA = Peroxy antioxidant compound

Synergistic antioxidants

Synergism defined as two or more antioxidants combine which provide more protection than would be predictable from the sum of that provided by the individual components. The stability of primary antioxidants will be improve by synergists which provide acidic medium. Both oxygen scavengers and chelating agents belong to this group. They act as various mechanisms.

Oxygen scavengers

The free oxygen group react by oxygen scavengers which end of results remove oxygen in closed system

Example: Sulfites, Ascorbic acid, Ascorbyl palminate, Erythorbic acid

Chelating agents

Chelators are not antioxidant but establish the chelating action when the molecular structure contain unshared pair of electron.Pro oxidant metals like iron and

copper and chelators react together to form stable complexes which promote and raise the energy of activation of the initiation reactions.

Example: Polyphosphates, EDTA, Tartaric acid, Citric acid

Secondary antioxidants

The lipid peroxides decomposing into stable products by secondary antioxidants.They are also called as preventive antioxidants. Secondary antioxidant consists of various trivalent phosphorous and divalent sulphur containing compounds.

Example: Thiodipropionic acid, Dilauryl, Distearyl esters

Miscellaneous antioxidant

The miscellaneous antioxidants such as flavonoids,amino acid,zinc, β-Carotene,selenium act as different role like synergist,inhibits lipid per oxidation,prevent the formation of hydroproxides, preventing the accumulation of hydrogen peroxide respectively.

Sources of antioxidants

Natural and Synthetic antioxidants are available from various sources.

Natural antioxidants

Vegetables, fruits, grain cereals, legumes, nuts etc. have varying amount of antioxidants.Some natural antioxidants and their sources [43] are listed given below

S.NO	Antioxidants	Food Source
1	Carotenoids	Yellow/orange vegetables and fruits, dark green leafy vegetables
2	Flavonoids	Vegetables and citrus fruits.
3	Isoflavones	Soyabeans.
4	Phenols.	Fruits and vegetables, green tea, wine
5	sterols.	Soybean
6	Protease inhibitor	Seeds and legumes

Synthetic antioxidants

Synthetic antioxidants are mainly phenolic and include butylated hydroxyl anisole(BHA), butylated hydroxyl toluene(BHT), tert-butyl hydro quinone(TBHQ) and propyl, octyl and dodecyl gallates.Polymeric antioxidants such as Anoxomer, Ionox–330, and Ionox–100, a derivative of BHT but they are not being used commercially.

Uses of antioxidants.

- Anti oxidants used for treatments of stroke and neurodegenerative diseases. such as Alzheimer's disease, Parkinson's disease[45-46]

- Antioxidants prevent the cell-damaging by free radicals[47]

- Rich source of antioxidants vegetables, and fruits help to lower risk of heart disease and some neurological diseases[48]

- Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health.

- Some evidence proven antioxidants source having vegetables and fruits who consume protect against a number of cancers[49]

- Antioxidants also used for preservatives in food and cosmetics material in industry.

- Antioxidant used for preventing the degradation of rubber and gasoline[51].

- It is used to prevent oxidation of fuels and lubricants[50].

MICRO ORGANISM

Microbes are tiny organisms,too tiny to see without a microscope yet they are rich on earth.They live everywhere like air,soil,rock,water,poles,deserts& deep-sea.Study of microorganisms is called Microbiology. Study of bacteria and viruses is called Bacteriology and virology respectively.Microorganisms play impartant role to humans and they contribute recycling other organisms and decomposition the waste products. some further advantageous activities of microbes are:

Use in Food:

Microbes are used in baking,other food making processes and also used the fermentation process in the production of dairy products like cheese.

Use in Science

Microbes are also vital tools in biotechnology,biochemistry,genetics and molecular biology.

Human digestion

The bacteria that live within the human digestive system supply to gut immunity, synthesizes vitamins and ferment complex indigestible carbohydrates.

In Medicines

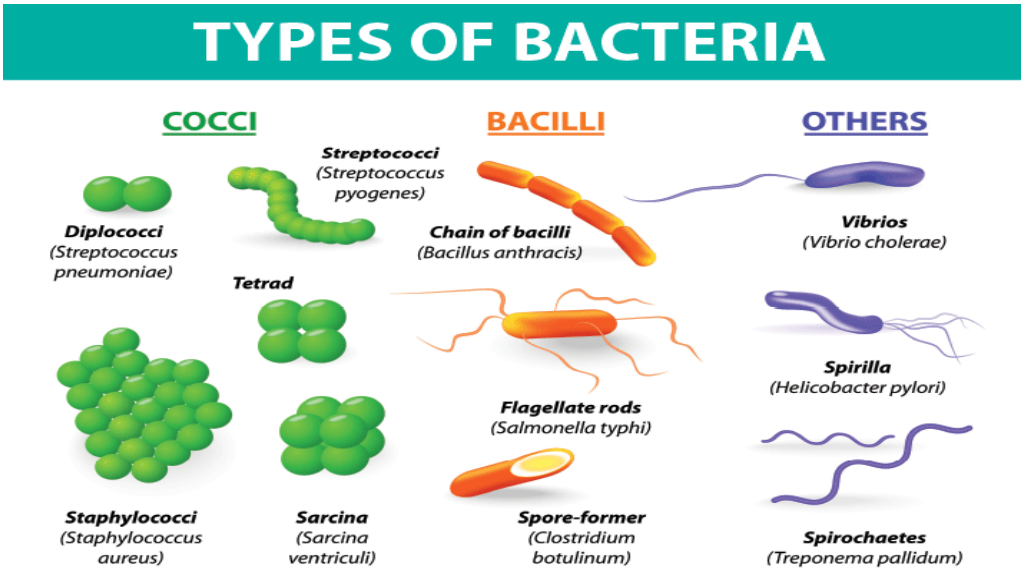
Microbes are used to make vaccines which can be stimulate the production of antibodies substances to make sure future defense against with unwanted microbes.

Most important types of microbes are Bacteria,Viruses,Fungi,Protozoa

Bacteria

Bacteria constitute a large domain of prokaryotic microorganisms. Typically a few micrometer in length, bacteria have a number of shapes including balls, commas, rods, cubes and spirals.These are very useful in many fields like preparation of antibiotics,in human digestion,in fermentation etc.

But they are spread out many infectious disease and which are main reason for universal mortality.

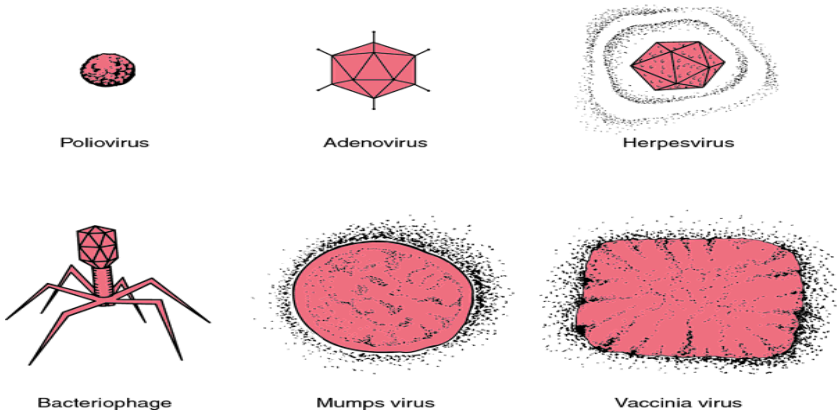


Some example for bacterial diseases are:

Gonorrhea, Syphilis, Anthrax, Tuberculosis, Cholera, Typhoid,fever,Pneumonia, Tetanus

Virus

A virus is a small infectious agent that multiply only inside the living cells of other organisms.These are among the smallest microbes than bacteria.It consists genes which are present in one or more molecules of DNA or RNA which surrounded by a protein coat.



Some example for viral diseases are:

Flu, Chickenpox, Polio, Rabies, Hepatitis A, Hepatitis B, Hepatitis C, AIDS

Antimicrobial

An antimicrobial is an agent which kills microorganisms or inhibits their growth.Antimicrobial can be classified intotwo types depends upon their function. Microbicidal which means antimicrobial agents that kill microbes while inhibit their growth are called biostatic.

Mechanism of action of antibiotics

Different antibiotics have different modes of action, due to the nature of their structure and degree of affinity to certain target sites within bacterial cells.

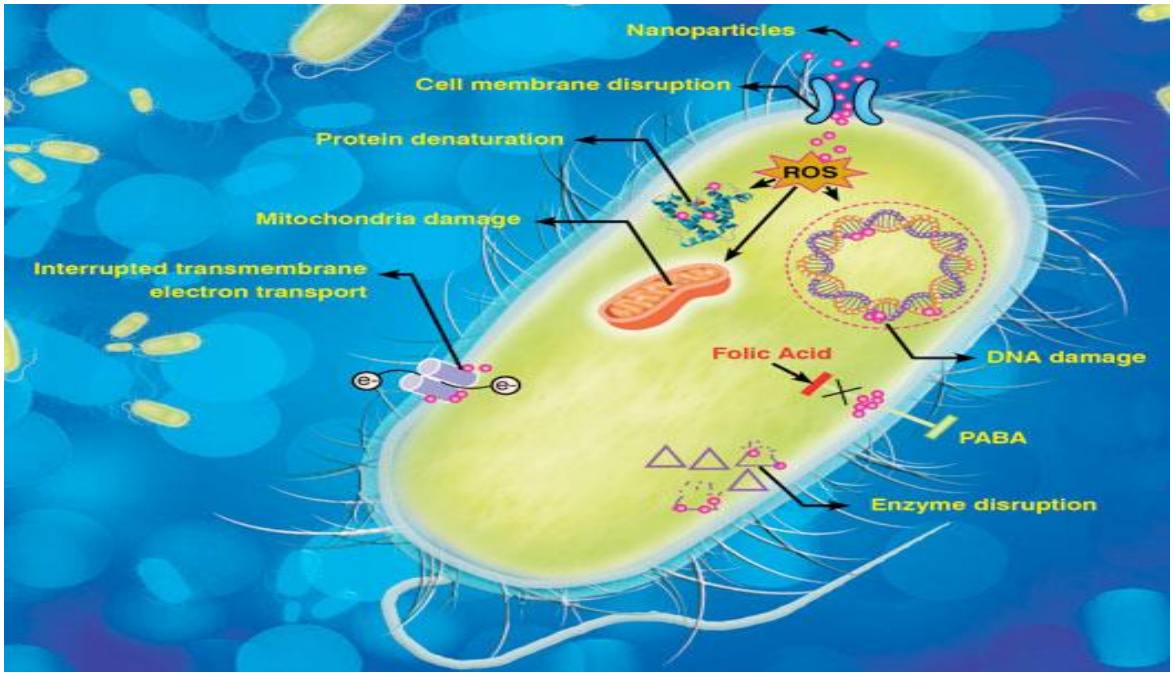
1.Cell wall synthesis

2.Protein synthesis

3.Cytoplasmic membrane permeability

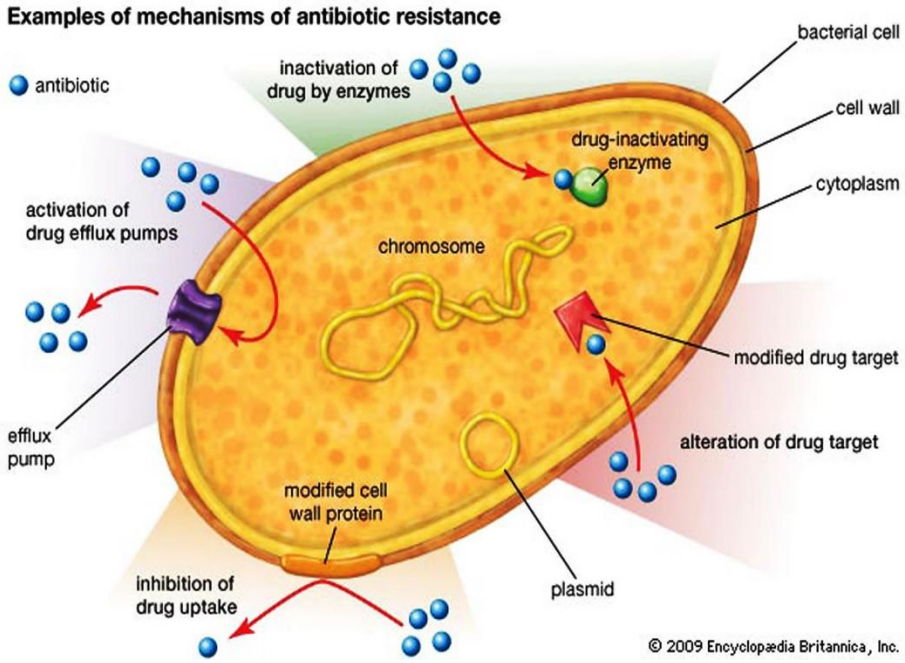
4.Nucleic acid synthesis

5.Anti metabolic synthesis



Antibiotic resistance

Antibiotic resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections. The bacteria survive and continue to multiply causing more harm. Bacteria can do this through several mechanisms.



LITERATURE REVIEW

LITERATURE REVIEW

Literature review related to antimicrobial activity:-

P. M Shafi *et al***, 2002** investigated the leaf essential oils of *Syzygium cumini* and *Syzygium travancoricum* were tested for their antibacterial property. The activity of *S. cumini* essential oil was found to be good, while that of *S. travancoricum* was moderate**.**

Silva, M. L. A. *et al***, 2007** reported the antimicrobial activity of *Syzygium cumini* leaves extract, known as "jambolão", was evaluated. The crude hydroalcoholic extract was active against *Candida krusei* (inhibition zone of 14.7 ± 0.3 mm and MIC = 70 µg / mL), and against multi–resistant strains of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*.

Satish. S *et al* , **2007** investigated aqueous extract of fifty-two plants from different families were tested for their antifungal potential against eight important species of Aspergillus such as A. candidus, A. columnaris, A. flavipes, A. flavus, A. fumigatus, A. niger, A. ochraceus, and A. tamarii which isolated from sorghum, maize and paddy seed samples. The test fungi were mainly associated with seed biodeterioration during storage. Among fifty-two plants tested, aqueous extract of Acacia nilotica, Achras zapota, Datura stramonium, Emblica officinalis, Eucalyptus globules, Lawsonia inermis, Mimusops elengi, Peltophorum pterocarpum, Polyalthia longifolia, Prosopis juliflora, Punica granatum and Sygigium cumini have recorded significant antifungal activity against one or the other Aspergillus species tested. A. flavus recorded high susceptibility and hence solvent extracts viz., petroleum ether, benzene, chloroform, methanol and ethanol extracts of all the twelve plants were tested for their antifungal activity against it. Among the solvent extracts tested, methanol gave more effective than ethanol, chloroform, benzene and petroleum ether, except for Polyalthia longifolia, where petroleum ether extract recorded highly significant antifungal activity than other solvent extracts**.**

S. Shyamala *et al***, 2010** carried out on the crude methanol and aqueous extracts of the leaves of *Syzygium cumini* (L.) (MYRTACEAE). The antimicrobial activity of the extract was tested against standard strains and clinical isolates of

some bacteria using the disc diffusion method. Preliminary phytochemical studies revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins as the chemical class present in the extracts. The extracts showed inhibitory activity against clinical isolates of the gram negative bacteria such as *Salmonella enteritidis*, *Salmonella typhi*, *Salmonella typhi A, Salmonella paratyphi A, Salmonella paratyphi B, Pseudomonas aeruginosa and Escherichia coli.* Gram positive bacteria are *Bacillus subtilis, and Staphylococcus aureus.* The results showed that the methanol extracts was more potent than the aqueous extracts.

Hofling, J. F. *et al***, 2010** reported the increase in the resistance to antimicrobial drugs in use has attracted the attention of the scientific community, and medicinal plants have been extensively studied as alternative agents for the prevention of infections. The *Candida* genus yeast can become an opportunistic pathogen causing disease in immunosuppressive hosts. The purpose of this study was to evaluate dichloromethane and methanol extracts from *Mentha piperita, Rosmarinus officinalis, Arrabidaea chica, Tabebuia avellanedae, Punica granatum* and *Syzygium cumini* against *Candida* species through the analysis of Minimum Inhibitory Concentration (MIC). Results presented activity of these extracts against *Candida* species, especially the methanol extract

Yadav *et al***, 2011** investigated *Syzygium cumini* L. better known as Jamun belonging to the family Myrtaceae is identified to have antidiabetic, anti-inflammatory, anti-pyretic and anti-oxidant activities. Anticancer activity of *S. cumini* L. fruits has been demonstrated. However, anticancer activity of *S. cumini* seeds on various types of human cancers has not been explored much. The methanol fraction of ethanol extract from the seeds of *S. cumini* was found to have significant antibacterial activity. This bioactive fraction was further tested positive for its anticancer activity on various types of human cancer cell lines indicating its potency. Structural characterization of the bioactive fraction was achieved using analysis of high performance liquid chromatography, ultra violet and infra red spectrum.

Afify *et al***, 2011** investigated the acaricidal activity of different extracts from *Syzygium cumini* (*S. cumini*) (Pomposia) againsst *Tetranychus urticae* Koch (*T.*

urticae) and the biochemical changes in antioxidants enzymes. Six extracts of *S. cumini* (Pomposia) at concentrations of 75, 150 and 300µg/mL were used to control *T. urticae* (Koch). The ethanol extract showed the most efficient acaricidal activity agent against *T. urticae* (98.5%) followed by hexane extract (94.0%), ether and ethyl acetate extract (90.0%). The LC_{50} values of the promising extract were 85.0, 101.0, 102.0 and 98.0µg/mL, respectively. The activities of enzymes including ascorbate peroxidase (APX), peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) in susceptible mites were increased. The activities of all antioxidant enzymes reach the maximum value in mites at LC_{50} with ethanol and ethyl acetate extracts, respectively. The extract of *S. cumini* has acaricidal activity against *T. urticae*, and the ethanol extract is the most efficient

A. Meshram *et al,* **2011** reported antibacterial activity against *E. coli*, *B. subtilis, P. aeruginosa and S. aureus* and inhibitory effect on glucoamylase of ethanolic extracts isolated at different temperatures from seeds of *Syzygium cumini* was investigated in vitro. All four strains were observed with moderate to good antibacterial activity. The ethanolic extract isolated at 200C showed maximum inhibition (50%) of glucoamylase activity. Thus we report the ethanolic extract of *Syzygium cumini* seeds is antibacterial and also potent inhibitor of glucoamylase. Hence may be hypoglycemic function in diabetes type-2.

R. Bhusari *et al***, 2014** investigated the powder prepared from the jamun seeds mixed with a small piece of jaggery for relief from diarrhoea and dysentery. Antibacterial activity of Syzygium cumini L. seed extracts prepared in Chloroform, Petroleum ether and Ethanol was evaluated by disc diffusion method. The solvent extracts is exerted a broad spectrum of bacteriostatic action against different gram-positive and gram-negative bacteria. Maximum antibacterial activity was observed in the ethanol extract against E. coli. The seed extract is shown the high therapeutic value against pathogenic bacteria and documented for futures

Literature review related to antioxidant:-

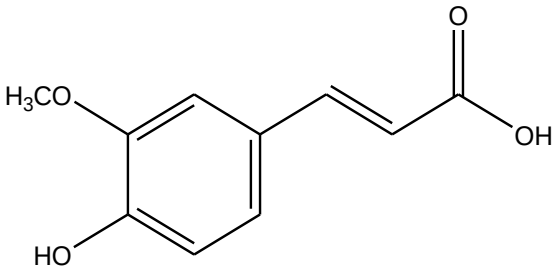
Veronica V *et al***, 2001** carried out meristematic cells of Allium cepa L. were used as vegetal test system and bone marrow cells of Wistar rats as animal test

system. Both were treated *in vivo* to evaluate whether the plants Averrhoa carambola L., *Syzygium cumini (L.)* Skeels and Cissus sicyoides L. presented cytotoxic and mutagenic effects and whether they resulted in cell alterations in their morphology, chromosomes or cell cycle division. Herbal teas were prepared as normally done by the population, albeit in two different concentrations, the usual concentration and a concentration ten times higher. Rats were treated with only one concentration of teas. Results showed that teas did not alter the cell cycle of Allium cepa L., with the exception of the 24 hours analysis after suspension of treatment (recovery of treatments), with a lower concentration of Averrhoa carambola. The latter had a low mitotic index when compared to control and to the post-treatment analysis, showing an inhibition of cell division. The three herbal teas neither induced an increase in the number of chromosomal damage in bone marrow cells of Wistar rats nor altered the cell division cycle. Results are important in so far as these plants are used as therapeutic agents.

A. Banerjee *et al***, 2004** reported food rich in antioxidants plays an essential role in the prevention of diseases. The fruits of wild Indian *Syzygium cumini* (L.) Skeels (Myrtaceae), also known as black plum, are edible. Traditionally they are also used to cure a number of ailments. In this paper, the antioxidant activity of the fruit skin has been analysed using different assays, such as hydroxyl radical-scavenging assay, based on the benzoic acid hydroxylation method, superoxide radical-scavenging assay, based on photochemical reduction of nitroblue tetrazolium (NBT) in the presence of a riboflavin-light-NBT system, DPPH radical-scavenging assay, and lipid peroxidation assay, using egg yolk as the lipid-rich source. Total antioxidant capacity was determined by the assay based on the reduction of Mo (VI)–Mo(V) by the extract and subsequent formation of a green phosphate/Mo (V) complex. In all the systems, a significant correlation existed between concentration of the extract and percentage inhibition of free radicals or percentage inhibition of lipid peroxidation. The antioxidant property of the fruit skin may come in part from the antioxidant vitamins, phenolics or tannins and anthocyanins present in the fruit

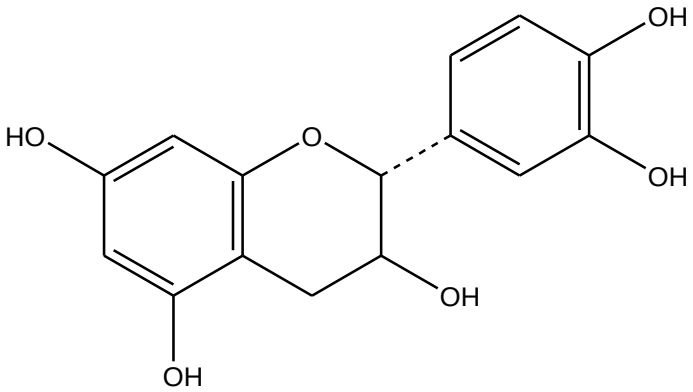
Z P Ruan *et al***, 2008** had performed the antioxidant activity of *Syzygium cumini* leaf extracts was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging and ferric-reducing antioxidant power (FRAP)

assays. The methanolic extract and its four water, ethyl acetate, chloroform, and n-hexane fractions were prepared and subjected to antioxidant evaluation. The results showed that the ethyl acetate fraction had stronger antioxidant activity than the other ones. HPLC data indicated that S. cumini leaf extracts contained phenolic compounds, such as ferulic acid (**1**) and catechin (**2**), responsible for their antioxidant activity. A significant linear relationship between antioxidant potency, free radical-scavenging ability and the content of phenolic compounds of leaf extracts supported this observation



(**1**)

Ferulic acid



(2)

catechin

M. Bajpai *et al***, 2005** studied to identify promising sources of antioxidants, some food and medicinal plants were studied for total phenolic contents and antioxidant activity. The leaves, bark and fruits of *Terminalia arjuna, Terminalia*

bellerica, Terminalia chebula and Terminalia muelleri, the leaves and fruits of *Phyllanthus emblica*, and the seeds of *Syzygium cumini* were found to have high total phenolic contents (72.0–167.2 mg/g) and high antioxidant activity (69.6–90.6%). Leaves of *Eucalyptus globulus* were a rich source of rutin, *Moringa oleifera* for kaempferol, aerial parts of *Centella asiatica* for quercetin, fruits of *T. bellerica* and *T. chebula* for gallic acid, and bark of *T. arjuna*, leaves and fruits of *T. bellerica* and bark, leaves and fruits of *T. muelleri* for ellagic acid

S. M. Hasan *et al***, 2009** carried out hydromethanol extracts of fifteen Bangladeshi medicinal plants, traditionally used in different ailments, were evaluated for antioxidant potential using DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging assay. Among the extracts *Cocos nucifera, Caesalpinia pulcherrima, Punica granatum* and *Syzygium cumini* were found displaying strong (90% or more) DPPH radical scavenging action. *Syzygium cumini* exhibited the highest radical scavenging, with an IC_{50} value of 4.25 µg/ml compared to the IC_{50} value of 5.15 µg/ml as shown by the reference antioxidant ascorbic acid, in a dose dependent fashion.

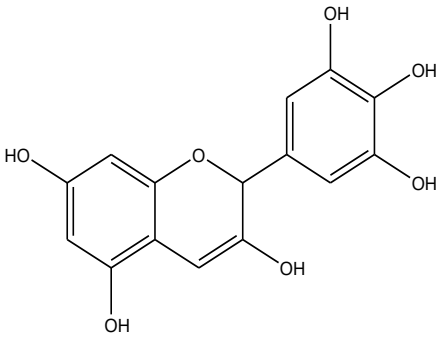
A. F. Faria *et al*, **2011** investigated the composition of carotenoids and phenolic compounds from jambolão fruits (*Syzygium cumini*) was determined by HPLC-DAD-MS/MS. Two main carotenoids were found in the fruits, all-*trans*-lutein (43.7%) and all-*trans*-β-carotene (25.4%). The anthocyanin composition was characterised by the presence of 3,5-diglucosides of five out of six aglycones commonly found in foods. This pattern was also observed for the other flavonoids, since diglucosides of dihydromyricetin, methyl-dihydromyricetin and dimethyl-dihydromyricetin, along with myricetin glucoside and a galloyl-glucose ester were identified. Furthermore, the antioxidant capacity of a functional extract rich in anthocyanins was evaluated through the scavenging capacities of $ABTS^{+}$ and peroxyl radical (ORAC) and the protective effect against singlet oxygen (O_2). The TEAC values indicated that the hemiacetals / chalcones and quinonoidal bases species ($pH \geqslant 5$) possess higher scavenging capacity as compared to the flavylium cation ($pH < 3$). The functional extract also showed 60% of dimethylanthracene protection against O_2 and an ORAC value of 16.4 µmol Trolox/g fruit.

Lekha K Nair *et al***, 2011** had performed *in vitro* antioxidant activity of the seed and leaf extract of Syzygium cumini. The antioxidant activity was determined by *in vitro* methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, ABTS Assay, Total antioxidant activity (Phosphomolybdic acid method), Nitric oxide radical scavenging, Ferric reducing antioxidant power (FRAP) assay, Hydroxyl radical scavenging activity, Total Reducing antioxidant potential, Reducing power. The extract showed significant antioxidant activity in all antioxidant assays when compared to ascorbic acid. The results of this research work are promising thus indicating the utilisation of the seed and leaf of *Syzygium cumini* as a significant source of natural antioxidants

Elizabeth Margaret *et al***, 2012** evaluated of antioxidant activity in methanolic extract of leaves, fruit pulp and seeds of *Syzygium cumini* (L.). Another aspect of the study was to evaluate the anti microbial activity of the different parts of the plant, keeping in view its pharmacological potential. The quantitative determination of compounds viz., phenolics and flavonoids supposed to be antioxidants was made and overall antioxidant activity was measured using standard methods. The results demonstrate that the total phenolic and flavonoid content of *S. cumini* leaves is greater than the content found in pulp and seed extracts. A linear correlation between total phenolic content and antioxidant activity (r2= 0.464529) has been reported. The results suggested that the phenolic compounds contribute effectively to the antioxidant activity. The highest antioxidant property of leaves is note worthy as compared to seed and pulp in the present study. Our study showed antibacterial activity against both the gram negative (*E. coli*) and gram- positive (*Staphylococcus aureus*) cultures grown in the leaf extract of *S. cumini*.

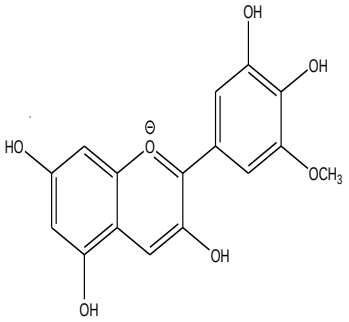
Gitanjali Tripathy *et al***, 2015** studied different concentrations of the methanolic extract of fruit pulp of the plant *Syzygium cumini* was subjected to *in-vitro* cytotoxic activity study against MCF-7cells using the MTT assay. Percentage cell viability of cell lines were carried out by using Trypan blue dye exclusion technique MTT assay was used to evaluate the reduction of viability of cell cultures in the presence and absence of the extract. Cell viability was inhibited to different extents by different concentrations of the extract.

J. M. Veigas *et al*, **2015** reported anthocyanin pigments from *Syzygium cumini* fruit peels were characterized and evaluated for their antioxidant efficacy, and stability as extract and in formulation. Total anthocyanin content was 216 mg/100 ml of extract which is equivalent to 230 mg/100 g fruit on a dry weight basis. Three anthocyanins were identified as glucoglucosides of delphinidin (**3**), petunidin (**4**) and malvidin (**5**) by HPLC–ESIMS. The antioxidant capacity of the extract was tested using models, such as DPPH-scavenging, reducing power assay, lipid peroxidation in rat brain, liver, liver mitochondria, testes and human erythrocyte ghosts. The extract showed 78.2% DPPH-scavenging at 2.5 ppm, while BHA exhibited only 41.6% activity at the same concentration, thus proving it to be a more efficient free radical-scavenger than the widely used BHA. One ppm of the extract was equivalent to 3.5 μM ascorbic acid, as estimated by reducing power assay. Inhibition of rat brain lipid peroxidation was 94.4% at 5.0 ppm concentration. It was almost equally active in all the biological models, except human erythrocyte ghost cells, where it showed only 48% inhibition at 5.0 ppm. The extract was quite stable at 0 °C with 11% loss in 4 weeks, while the pigment loss in the antitussive formulation was only 13% at 30 °C at the end of 8 weeks. The high antioxidant activity and relatively high stability of the pigments make *S. cumini* a potential source of natural colourant as well as antioxidants



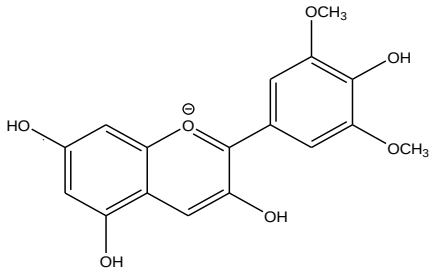
(**3**)

Delphinidin.



(**4**)

Petunidin



(**5**)

Malvidin

Literature review related to antidiabetic:-

Pandey *et al***, 2002** had reported the effect of feeding orally along with diet of different fractions obtained from the seeds of *S. cumini* was tried on fasting blood glucose and glucose tolerance in normal and alloxan diabetic rats. The quantitative determination showed that S. *cumini* seeds contained 40% of water soluble gummy fibre and 15% of water insoluble neutral detergent fibre (NDF). This study demonstrated that feeding for 21 days of the diets containing 15% powdered unextracted (intact) seeds containing water soluble gummy fibre, 15% powdered defatted seeds from which lipid and saponins were removed only and 6% water soluble gummy fibre isolated from S. *cumini* seeds significantly lowered blood glucose levels and improved oral glucose tolerance whereas feeding of the diets containing 15% powdered degummed S. *Cumini* seeds from which water soluble gummy fibre was removed but which contained neutral detergent fibre(NDF)and 2.25% water insoluble neutral detergent fibre (NDF) isolated from S. *cumini* seeds neither lowered blood glucose levels nor improved oral glucose tolerance in both normal and diabetic rats. These observations indicate that the hypoglycaemic effect of S. *cumini* seeds was due to water soluble gummy fibre and also that water insoluble neutral detergent fibre (NDF) and other constituents of the seeds had no significant hypoglycaemic effects.

I. M. Villaseñor *et al,* **2005** had reported validation of the ethnobotanical use of the leaves of *Artemisia vulgaris* Linn. (*Compositae*), *Eucalyptus tereticornis* Sm. (*Myrtaceae*), *Solanum nigrum* Linn. (*Solanaceae*) and *Vitex negundo* Linn.

(*Verbenaceae*); stems of *Nopalea cochinellifera* (Linn.) Salm-Dyck (*Cactaceae*); roots of *Imperata cylindrica* Beauv. (*Gramineae*) dried bark of *Syzygium cumini* (Linn.) Skeels (*Myrtaceae*) as anti-diabetic agents using the oral glucose tolerance test showed that only the bark of *Syzygium cumini* and the leaves of *Vitex negundo* and *Eucalyptus tereticornis* exhibited anti-hyperglycemic activities when fed simultaneously with glucose. At the same dosages of 5 mg/20 g mouse, *Syzygium cumini*-treated mice showed a significant decrease in blood glucose levels (BGLs) at 30 min ($\alpha = 0.10$) and from 45 min onwards at $\alpha = 0.05$. *Vitex negundo* exhibited greater anti-hyperglycemic activity than *Eucalyptus tereticornis*. Both showed a significant decrease in BGLs at 60 min but at$\alpha = 0.05$ for *Vitex negundo* and at $\alpha = 0.07$ for *Eucalyptus tereticornis*. There was no significant lowering in BGLs for *Imperata cylindrica* and *Solanum nigrum* while there was even an increase in BGLs for *Nopalea cochinellifera* and *Artemisia vulgaris*

R. Anandharajan *et al* **, 2006** carried out the purpose of the present study is to investigate the effect of methanolic extracts of *Aegles marmelos* and *Syzygium cumini* on a battery of targets glucose transporter (Glut-4), peroxisome proliferator activator receptor gamma (PPARγ) and phosphatidylinositol 3′ kinase (PI3 kinase) involved in glucose transport. *A. marmelos* and *S. cumini* are anti-diabetic medicinal plants being used in Indian traditional medicine. Different solvent extracts extracted sequentially were analysed for glucose uptake activity at each step and methanol extracts were found to be significantly active at 100 ng/ml dose comparable with insulin and rosiglitazone. Elevation of Glut-4, PPARγ and PI3 kinase by *A. marmelos* and *S. cumini* in association with glucose transport supported the up-regulation of glucose uptake. The inhibitory effect of cycloheximide on *A. marmelos*- and *S. cumini*-mediated glucose uptake suggested that new protein synthesis is required for the elevated glucose transport. Current observation concludes that methanolic extracts of *A. marmelos* and *S. cumini* activate glucose transport in a PI3 kinase-dependent fashion.

J. Shinde *et al***, 2008** had performed *Syzygium cumini* seed kernel extracts were evaluated for the inhibition of α-glucosidase from mammalian (rat intestine), bacterial (*Bacillus stearothermophilus*), and yeast (*Saccharomyces cerevisiae*, baker's yeast). In vitro studies using the mammalian α-glucosidase from rat intestine

showed the extracts to be more effective in inhibiting maltase when compared to the acarbose control. Since acarbose is inactive against both the bacterial and the yeast enzymes, the extracts were compared to 1-deoxynojirimycin. We found all extracts to be more potent against α-glucosidase derived from *B. stearothermophilus* than that against the enzymes from either baker's yeast or rat intestine. In an in vivo study using Goto–Kakizaki (GK) rats, the acetone extract was found to be a potent inhibitor of α-glucosidase hydrolysis of maltose when compared to untreated control animals. Therefore, these results point to the inhibition of α-glucosidase as a possible mechanism by which this herb acts as an anti-diabetic agent.

A. Kumar *et al***, 2008** investigated *Syzygium cumini* (Myrtaceae) is widely used traditional system of medicine to treat diabetes in India. The present study was carried out to isolate and identify the putative antidiabetic compound from the *S. cumini* [SC] seed. A compound, mycaminose was isolated from SC seed extract. The isolated compound mycaminose (50 mg/kg) and ethyl acetate [EA] and methanol [ME] extracted compounds of *S. cumini* seed (200 and 400 mg/kg) was undertaken to evaluate the anti-diabetic activity against streptozotocin (STZ)-induced diabetic rates. The compound 'Mycaminose' and ethyl acetate and methanol extracted produced significant ($p<0.05$) reduction in blood glucose level. The standard drug, glibenclamide (1.25 mg/kg) also produced significant ($p<0.05$) reduction in blood glucose level against STZ-induced diabetic rats. The results of this experimental study indicate that isolated compound 'Mycaminose', ethyl acetate and methanol extracts possess anti-diabetic effects against STZ**-**induced diabetic rats.

A. Bopp *et al***, 2009** had reported Syzigium cumini (L.) Skeels from the Myrtaceae family is among the most common medicinal plants used to treat diabetes in Brazil. Leaves, fruits, and barks of S. cumini have been used for their hypoglycemic activity. Adenosine deaminase (ADA) is an important enzyme that plays a relevant role in purine and DNA metabolism, immune responses, and peptidase activity. ADA is suggested to be an important enzyme for modulating the bioactivity of insulin, but its clinical significance in diabetes mellitus (DM) has not yet been proven. In this study, we examined the effect of aqueous leaf extracts of S. cumini (L.) (ASC) on ADA activity of hyperglycemic subjects and the activity of total ADA, and its isoenzymes in serum and erythrocytes. The present study indicates

that: (i) the ADA activity in hyperglycemic serum was higher than normoglycemic serum and ADA activity was higher when the blood glucose level was more elevated; (ii) ASC (60–1000 μg/mL) in vitro caused a concentration-dependent inhibition of total ADA activity and a decrease in the blood glucose level in serum; (iii) ADA1 and 2 were reduced both in erythrocytes and in hyperglycemic serum. These results suggest that the decrease of ADA activity provoked by ASC may contribute to control adenosine levels and the antioxidant defense system of red cells and could be related to the complex ADA/DPP-IV-CD26 and the properties of dipeptidyl peptidase IV (DPP-IV) inhibitors which serve as important regulators of blood glucose.

Silva, A. *et al***, 2009** had performed the effects of prolonged treatment with *Syzygium cumini* (Jambolan) sheet aqueous extract on the structure of cells responsible for secretory processes in the parotid and submandibular salivary glands of spontaneously diabetic mice. Ten female mice, including five diabetic Nod mice (group I) and five BALB/c mice (group II), were used. After characterization of the diabetic state, animals of group I received *Syzygium cumini* extract and group II animals received water *ad libitum*. After the experimental period, the salivary glands were collected from the animals for stereological analysis. The results showed structural alterations in the salivary glands of diabetic animals characterized by nuclear and cytoplasmic atrophy and the occurrence of inflammatory cells, as well as elevated glycemia levels. We conclude that no recovery of normal glycemia levels or glandular tissue structure occurs in diabetic animals even when treated with *Syzygium cumini* extract, a fact that might result in changes in the functional mechanisms of these organs

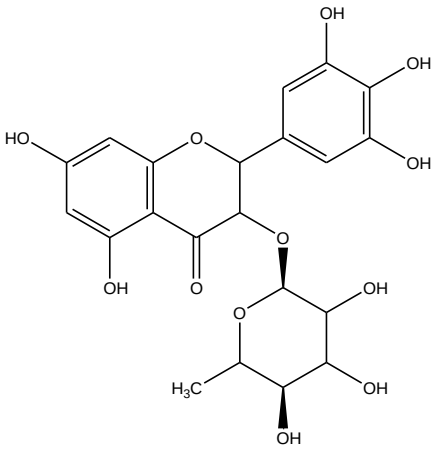
L Nahar *et al***, 2010** had reported herbal formulations are getting more importance in the treatment of diabetes, cancer and hepatic disorder because of the hazardous adverse effects of the current therapy. Especially diabetes can be controlled by Allopathic medicine as well as Herbal medicine. This study would be helpful for the industry to produce herbal formulation with less side effects and cost effective treatment for diabetes. Moreover in vivo models were utilized to test the antidiabetic property and it will be useful for fix the optimized dose in herbal formulation. The findings of the bioactive molecule from the herbals would be effective drug target against the diabetes. A comparison was made between the

antidiabetic activities of methanolic extracts of leaves of *Abroma augusta* and seeds of *Syzygium cumini* in alloxan induced diabetic rats. Serum glucose level, weight variation and other histopathological studies were conducted at the dose 300 mg/kg body weight for 7 days. There was a significant decrease in serum glucose level ($p<0.001$), increase in body weight and changes in normal cells was observed with treatment of the above mentioned extracts which altered in diabetic rats as compared to the standard.

K Bona *et al***, 2011** had performed the effects of *Syzygium cumini* leaf extract (ASc), on Adenosine deaminase (ADA) and Acetyl cholinesterase (AChE) activities, and also on oxidative stress parameters in erythrocytes hemolysates (RBCs) and erythrocytes membranes (ghosts) from type 2 diabetics patients (Type 2 DM) under *in vitro* conditions. Non protein thiol groups (NP-SH), AChE, Catalase (CAT) and Superoxide Dismutase (SOD) activities were measure in RBCs. Further, ADA activity, Thiobarbituric Acid-Reactive Substances (TBARS) levels and protein thiol groups (P-SH) were estimated in ghosts. Also, P-SH and Vitamin C (VIT C) were measure in plasma sample. The results demonstrated that ADA and AChE activities, besides TBARS levels were higher in erythrocytes of Type 2 DM, while SOD activity and NP-SH levels were decreased when compared to control group. ASc, *in vitro*, reduced ADA and AChE activities and some parameters of oxidative stress. Furthermore, we observed correlations between VIT C and P-SH levels, ADA activity and P-SH levels, as well as NP-SH and TBARS levels in diabetics. The results suggest that ASc *in vitro* is able to promote the reduction of inflammation and oxidative stress parameters, and act against biochemical changes occurring in Diabetes *mellitus* (DM).

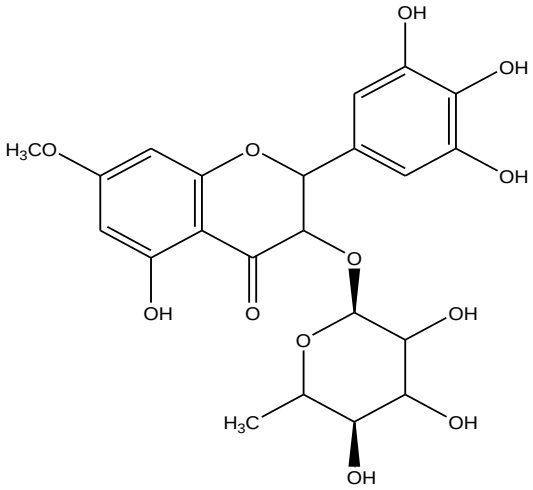
T. Manaharan *et al*, **2016** carried out *Syzygium aqueum* is a medicinal plant which is grown in tropical regions. In this study, the ethanolic extracts of *S. aqueum* leaf were investigated for its anti hyperglycaemic activity. Our investigation revealed its effectiveness in inhibiting the carbohydrate hydrolysing enzymes, α-glucosidase (EC_{50} = 11 µg/ml) and α-amylase (EC_{50} = 8 µg/ml), at significant level than the commercial drug acarbose (EC_{50} = 28 µg/ml, α-glucosidase; EC_{50} = 12 µg/ml, α-amylase). In addition, the ethanolic leaf extracts were able to inhibit the key enzyme in the polyol pathway, aldose reductase (EC_{50} = 0.03 µg/ml) and prevent the AGEs

formation by 89%. Six flavonoid compoundsmyricetin-3-*O*-rhamnoside (6), europetin-3-*O*-rhamnoside (**7**), phloretin (**8**), myrigalone-G(**9**) and myrigalone-B(1**0**), 4-hydroxybenzaldehyde(**11**),were isolated from the ethanolic leaf extracts. Compounds and showed high inhibitory activities, with EC_{50} values of 1.1 µM and 1.9 µM against α-glucosidase and EC_{50} values of 1.9 µM and 2.3 µM against α-amylase, respectively. These findings provide a strong rationale to establish *S. aqueum*'s capability as an anti hyperglycaemic agent



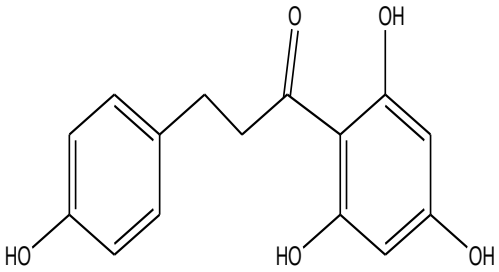
(**6**)

Myricetin-3-*O*-rhamnoside



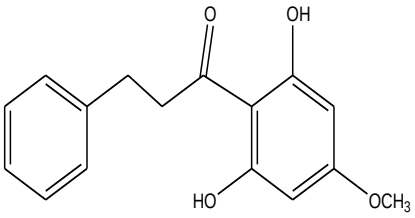
(**7**)

Europetin-3-*O*-rhamnoside



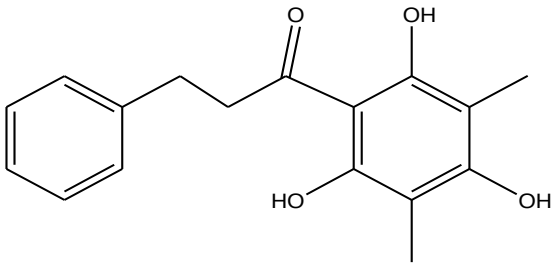
(**8**)

Phloretin



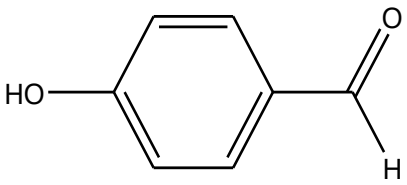
(**9**)

Myrigalone-G



(**10**)

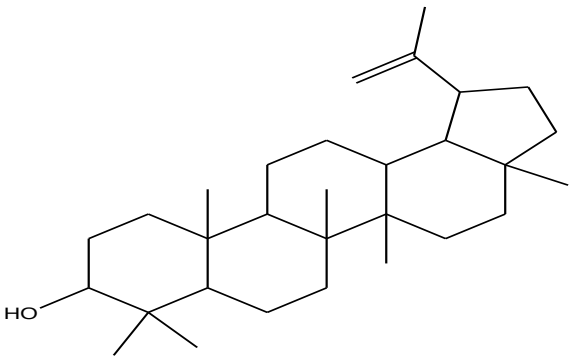
Myrigalone-B



(**11**)

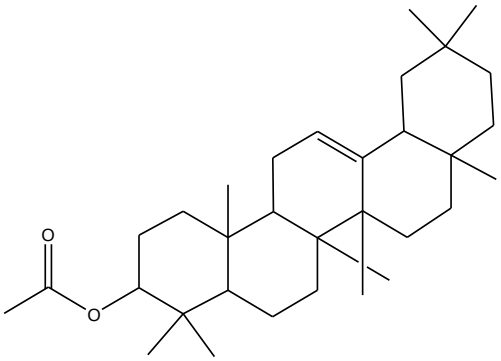
4-hydroxybenzaldehyde

Alam *et al***, 2012** investigated traditionally the leaves of Syzygium cumini (Myrtaceae) are widely used for treating diabetes. The present study was carried out to identify the putative antidiabetic constituents from the *S. cumini* leaves. From the NMR data four different compounds, Lupeol (**12**), 12-oleanen-3-ol-3β-acetate (**13**), Stigmasterol(**14**), βsitosterol(**15**) were identified from n-hexane fraction of plant extract. These compounds have potential antidiabetic activities which support the traditional use of the leaves as being remedy for treating diabetes



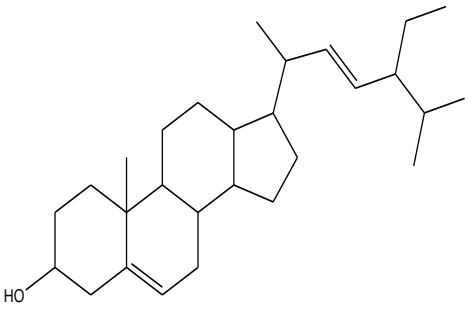
(**12**)

Lupeol



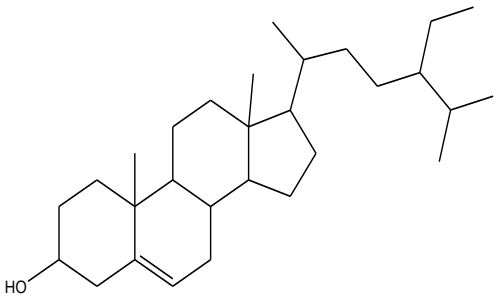
(**13**)

. 12-oleanen-3-ol-3β-acetate



(**14**)

Stigmosterol



(**15**)

βsitosterol

C.C. Teixeira *et al***, 2007** investigated a tea prepared from *Syzygium cumini* (L.) Skeels, reported to be used by diabetics in Porto Alegre, Brazil, might have an antihyperglycemic effect in experimental models. Teas prepared from leaves and seeds of *S. cumini*, in concentrations ranging from 2–64 g/l, were administered, as water substitute for 14–95 days, to 16 groups with 8–9 normal albino rats and to four groups with 10–12 rats with streptozotocin-induced diabetes mellitus. Post-prandial blood glucose levels were determined by the glucose oxidase method on blood samples obtained by decapitation. None of the tea concentration had any detectable antihyperglycemic effect either in normal or in diabetic rats, suggesting that this plant, prepared in a manner similar to that employed by humans, is destitute of an antihyperglycemic effect.

P. Oliveir *et al*, **2010** investigated the effects of extracts and fractions of *Baccharis trimera* and *Syzygium cumini* on glycaemia of diabetic and non-diabetic mice. Crude ethanolic extracts and aqueous and butanolic fractions of the aerial parts *of Baccharis trimera* and leaves of *Syzygium cumini* were evaluated. None of the extracts or fractions (200 or 2000 mg/kg, per os) induced any effect after acute administration. Seven-day treatment with crude ethanolic and aqueous and butanolic fractions (200–2000 mg/kg, twice daily, per os) of *Syzygium cumini* reduced glycaemia of non-diabetic mice. However, this effect was associated with a reduction of food intake and body weight, indicating that this may not be a genuine hypoglycaemic effect. In diabetic mice, only the aqueous fraction of *Baccharis*

trimera (2000 mg/kg, twice daily, per os) reduced the glycaemia after a 7-day treatment. This effect was not associated with a body weight reduction. The results suggest that *Baccharis trimera* presents a potential antidiabetic activity and indicate that food intake and body weight must be determined when evaluating metabolic parameters after prolonged administration of plant extracts**.**

A. Helmstadter *et al*, **2010** had reported *Syzygium cumini* (L.) Skeels (syn. *S. jambolanum* DC, *Eugenia jambolana* Lam.) belongs to the medicinal plants most often recommended as an adjuvant therapy in type 2 diabetes. The plant was extensively studied during the last 125 years, approximately 100 case reports were reported already before the discovery of insulin. After the Second World War, research was concentrated on animal studies. Not all, but many of them reported some success in reducing type 2 diabetes symptoms. However, a state-of-the-art clinical study is still missing. In this review, historical literature dating back to the pre-insulin era was evaluated as were more recent *in vitro*-, animal-, and *in vivo* studies. Results were screened for information still useful today and compared to study results achieved in more recent decades. In view of the knowledge summarized here, a successful clinical study should use *S. cumini* seeds, seed kernels or fruit from India in fairly high doses. Reductions on blood sugar levels by about 30% seem reasonably to be expected. Adverse effects to be expected comprise gastrointestinal disturbances

MISCELLANEOUS

S Muruganandan *et al***, 2001** had performed the ethanolic extract of the bark of *Syzygium cumini* (L.) Skeels was investigated for its anti-inflammatory activity in animal models. The extract did not show any sign of toxicity up to a dose of 10.125 g/kg, p.o. in mice. Significant anti-inflammatory activity was observed in carrageenin (acute), kaolin-carrageenin (subacute), formaldehyde (subacute)-induced paw oedema and cotton pellet granuloma (chronic) tests in rats. The extract did not induce any gastric lesion in both acute and chronic ulcerogenic tests in rats. Thus, the present study demonstrated that *S. cumini* bark extract has a potent anti-inflammatory action against different phases of inflammation without any side effect on gastric mucosa.

G. C. Jagetia *et al***, 2002** reported the effects of various concentrations (0.0, 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 µg/ml) of the leaf extract of *Syzygium cumini* Linn. or *Eugenia cumini* (SC; black plum, Jamun, family Myrtaceae) was studied on the alteration in the radiation-induced micronuclei formation in the cultured human peripheral blood lymphocytes. Treatment of lymphocytes to various concentrations of SC resulted in a dose dependent increase in the micronuclei-induction, especially after 25–100 µg/ml extract. The exposure of human lymphocytes to various concentrations of SC extract before 3 Gy γ-irradiation resulted in a significant decline in the micronuclei-induction at all the drug doses when compared with the non-drug treated irradiated cultures. A nadir in MNBNC frequency was observed for 12.5 µg/ml drug concentration, where the MNBNC frequency was approximately fourfold lower than that *oetf* the non-drug treated irradiated cultures. Therefore, this dose may be considered as an optimum dose for radiation protection. Our study demonstrates that the leaf extract of *S. cumini*, a plant traditionally used to treat diabetic disorders protects against the radiation-induced DNA damage.

P. King *et al***, 2007** had perform the biosorption of lead ions from aqueous solution by *Syzygium cumini* L. was studied in a batch adsorption system as a function of pH, contact time, lead ion concentration, adsorbent concentration and adsorbent size. The biosorption capacities and rates of lead ions onto *S. cumini* L. were evaluated. The Langmuir, Freundlich, Redlich–Peterson and Temkin adsorption models were applied to describe the isotherms and isotherm constants. Biosorption isothermal data could be well interpreted by the Langmuir model followed by Temkin model with maximum adsorption capacity of 32.47 mg/g of lead ion on *S. cumini* L. leaves biomass. The kinetic experimental data were properly correlated with the second-order kinetic model.

Rakesh Kumar *et al*, **2007** investigated tannase producing fungal strains were isolated from different locations including garbages, forests and orchards, etc. The strain giving maximum enzyme yield was identified to be *Aspergillus ruber*. Enzyme production was studied under solid state fermentation using different tannin rich substrates like ber leaves (*Zyzyphus mauritiana*), jamun leaves (*Syzygium cumini*), amla leaves (*Phyllanthus emblica*) and jawar leaves (*Sorghum vulgaris*). Jamun leaves were found to be the best substrate for enzyme production under

solid-state fermentation (SSF). In SSF with jamun leaves, the maximum production of tannase was found to be at 30 °C after 96 h of incubation. Tap water was found to be the best moistening agent, with pH 5.5 in ratio of 1:2 (w/v) with substrate. Addition of carbon and nitrogen sources to the medium did not increase tannase production. Under optimum conditions as standardized here, the enzyme production was 69 U/g dry substrate. This is the first report on production of tannase by *A. ruber*, giving higher yield under SSF with agro-waste as the substrate.

F.A. Brito *et al***, 2007** carried out Myrtaceae is a plant family widely used in folk medicine and *Syzygium* and *Eugenia* are among the most important genera. We investigated the anti-allergic properties of an aqueous leaf extract of *Syzygium cumini* (L.) Skeels (SC). HPLC analysis revealed that hydrolyzable tannins and flavonoids are the major components of the extract. Oral administration of SC (25-100 mg/kg) in Swiss mice (20-25 g; N = 7/group) inhibited paw edema induced by compound 48/80 (50% inhibition, 100 mg/kg; P £ 0.05) and, to a lesser extent, the allergic paw edema (23% inhibition, 100 mg/kg; P £ 0.05). SC treatment also inhibited the edema induced by histamine (58% inhibition; P £ 0.05) and 5-HT (52% inhibition; P £ 0.05) but had no effect on platelet-aggregating factor-induced paw edema. SC prevented mast cell degranulation and the consequent histamine release in Wistar rat (180-200 g; N = 7/group) peritoneal mast cells (50% inhibition, 1 µg/mL; P £ 0.05) induced by compound 48/80. Pre-treatment of BALB/c mice (18-20 g; N = 7/group) with 100 mg/kg of the extract significantly inhibited eosinophil accumulation in allergic pleurisy (from 7.662 ± 1.524 to 1.89 ± 0.336 x 10^6/cavity; P £ 0.001). This effect was related to the inhibition of IL-5 (from 70.9 ± 25.2 to 12.05 ± 7.165 pg/mL) and CCL11/eotaxin levels (from 60.4 ± 8.54 to 32.8 ± 8.4 ng/mL) in pleural lavage fluid, using ELISA. These findings demonstrate an anti-allergic effect of SC, and indicate that its anti-edematogenic effect is due to the inhibition of mast cell degranulation and of histamine and serotonin effects, whereas the inhibition of eosinophil accumulation in the allergic pleurisy model is probably due to an impairment of CCL11/eotaxin and IL-5 production.

A. Kumar *et al*, **2007** had perform the Syzygium cumini (Myrtaceae) is a traditional medicine plant for the treatment of hypoglycemic, antibacterial, anti-HIV and antidiarrheal activities. The work reached the acute toxicity of Syzygium cumini

and its action on the Central Nervous System (CNS) because no data in the literature have been found of pharmacological activity of this plant in the CNS. The seed was extracted with ethyl acetate and methanol and investigated for its Central Nervous System activity (CNS) of Albino mice in rota rod and actophotometer at the dose level of 200 mg/kg and 400 mg/kg. Both the extract exhibited significantly CNS activity. This study established CNS activity in Syzygium cumini seed.

J. M. Veigas *et al***, 2008** carried out *Syzygiumcumini*, Indian black plum or Java plum, is a rich source for anthocyanins (230 mg/100 g DW) showing high antioxidant activity *in vitro*. In the following study it is further demonstrated that *S. cumini* peel extract rich in anthocyanins (SCA) offers considerable protection against carbon tetrachloride (CCl_4)-induced damage in rat hepatocytes. SCA itself being non-toxic to primary rat hepatocytes at concentrations ranging from 50 to 500 ppm, was found to suppress CCl_4-induced LDH leakage by 54% at 50 ppm, thereby improving the cell viability by 39%. The SCA significantly reversed the CCl_4 induced changes in cellular glutathione (GSH) level, lipid peroxidation and activity of the antioxidant enzyme glutathione peroxidase. Exposure of hepatocytes to SCA after CCl_4 treatment was found to elevate GSH and GPx activities by 2-folds, whereas the activities of catalase and superoxide dismutase were not significantly affected. The fruit pulp extract (SPE) was less effective in offering protection to rat hepatocytes, particularly in terms of total GSH content and a consequent increase in lipid peroxidation although the higher GPx activity suggests the probable involvement of GSH as a substrate for GPx. These observations suggest that the fruit peel extract of *S. cumini*, is largely responsible for the reversal of CCl_4-induced oxidative damage in rat hepatocytes. Both peel and pulp extract appear to offer protection to rat hepatocytes through GPx along with other biological pathways independent of catalase and superoxide dismutase.

K.S. Rao *et al***, 2011** carried out *Syzygium cumini L* leaf powder was used as a biosorbent for generating adsorption data in a fixed bed mini column. Effect of flow rate, initial Cd(II) concentration and bed height were the experimental parameters chosen to obtain breakthrough curves. The maximum uptake of Cd(II) in a fixed bed adsorption column was 29.08 mg g^{-1} at pH 5.5, initial Cd(II) concentration 100 mg L^{-1}, bed height 5 cm and flow rate 40 mL min^{-1}. Bohart–Adams, BDST,

Thomas and Yoon–Nelson models were applied to the data for predicting breakthrough curves and to determine the characteristic parameters. Prominent and unique characteristics features of the respective models like service time (Hutchins BDST model), adsorption capacity (Thomas model) and time required for 50% breakthrough (Yoon–Nelson model) were determined. The utilization of column data in designing of a commercial column has been discussed. It was possible to desorb ~98% of Cd(II) using 0.05 N HCl solution in column.

B. Chaudhary *et al*, **2012** investigated *Syzygium cumini* (L.) Skeels, commonly known as Jamun, is a widely distributed forest tree in India and other tropical and sub tropical regions of the world. The tree has a great economic importance since most of the parts like the bark, leaves, seed and fruits are used as an alternative medicine to treat various diseases. It is used in well known traditional medicines to control the blood sugar level in the patients suffering from diabetes. The tree is rich in phytochemicals like glycoside jambolin, anthocyanins, tannins, terpenoids, gallic acid and various minerals. These wide ranges of health promoting compounds make them a suitable candidate to be used as a nutraceutical. The fruits are purplish black in colour when ripe and have high anthocyanin content. It is a seasonal fruit and is consumed fresh for its nutrient value. Fruits are also processed to make jam, jellies, squash, vinegar and ice cream for its beautiful and attractive purple colour. There are many commercial herbal brands in India and other Asian countries which manufacture these products and are very popular among the consumers. Even though there has been a number of successful research on the medicinal properties of S. cumini extracts in animal models and in vitro animal cell lines there are no reports on clinical trial experiments to study the in vivo effect of the phytochemicals on human beings. We suggest that there is a need to do further research and study the suitability of this tree extracts as a nutraceutical.

Rachel Melo Ribeiro *et al***, 2012** had performed *in vivo* potential antihypertensive effect of hydroalcoholic extract of *Syzygium cumini* leaves (HESC) in normotensive Wistar rats and in spontaneously hypertensive rats (SHR), as well as its *in vitro* effect on the vascular reactivity of resistance arteries. The hypotensive effect caused by intravenous infusion of HESC (0.01–4.0mg/kg) in anesthetized Wistar rats was dose-dependent and was partially inhibited by pretreatment with

atropine sulfate. SHR received HESC (0.5 g/kg/day), orally, for 8 weeks and mean arterial pressure, heart rate, and vascular reactivity were evaluated. Daily oral administration of HESC resulted in a time-dependent blood pressure reduction in SHR, with a maximum reduction of 62%. In the endothelium-deprived superior mesenteric arteries rings the treatment with HESC reduced by 40% the maximum effect (Emax) of contraction induced by NE. The contractile response to calcium and NE of endothelium-deprived mesenteric rings isolated from untreated SHR was reduced in a concentration-dependent manner by HESC (0.1, 0.25, and 0.5mg/mL). This study demonstrated that *Syzygium cumini* reduces the blood pressure and heart rate of SHR and that this antihypertensive effect is probably due to the inhibition of arterial tone and extracellular calcium influx.

Islam M *et al*, **2015** investigated the hepatoprotective effects of seeds of *Syzygium cumini* L. (Family: *Myrtaceae*), presumed to be effective in treating gastrointestinal diseases of animals, have not been investigated before, particularly in liver damage caused by infections, chemicals and xenobiotics. Therefore, this aimed at investigating the hepatoprotective effects of methanol extracts of plant seeds in chemically (CCl4) induced stress rats. Adult male, *Sprague Dawley* rats (n=30) were randomly segregated into 5 equal groups i.e., group-I (control), group-II (silymarin treated; 1.0 mg/kg BW), group-III (extract of *Syzygium cumini* seeds treated; 250 mg/kg BW), group-IV (extract treated; 500 mg/kg) and group-V (CCl4 treated; 1.5 mg/kg).Rats were treated with respective treatments for 14 consecutive days. At day 14, four hours after the last dose, an oral dose of CCl4 (1.5 mg/kg, 1:1 in olive oil) was administered to all the groups, except animals in the control group. Subsequently, 24h later, blood samples and liver tissues were collected for biochemical analysis and histopathology, respectively. The values of liver function markers were found to be significantly ($P<0.05$) lower while serum protein level was significantly higher in control and treated groups as compared to that of the CCl4 treated group. Histological examination of liver tissues also indicated that the extract of *Syzygium cumini* seeds in both the doses, and silymarin protected the liver from CCl4-induced stress. It was concluded that extract of seed of *Syzygium cumini* has hepatoprotective activity.

Deepti Katiyar *et al***, 2016** reported *Syzygium cumini* (Linn.) Skeels. (Myrtaceae) (Synonym. *Eugenia jambolana*), commonly known as Jamun or Black Plum is indigenous to India. The plant has traditionally been used for its rich nutrition and medicinal value. The current review focuses on the recent research investigations involving the studies on diverse pharmacological actions of Jamun. The electronic database search was performed in order to achieve this aim. Jamun plant has been reported to be enriched with an array of phytoconstituents like ellagic acid, glucoside, anthocyanins, kaempferol, isoquercetin, myrecetin and so on. The seeds which are mostly utilized for their medicinal value are said to contain an alkaloid- jambosine and glycoside-jambolin or antimellin which inhibit the conversion of starch to sugar. Different plant parts have been claimed to contain different constituents, due to which they possess an assorted pharmacological prospective. Jamun has been used for the treatment of diabetes since ages. Apart from this it has also shown its beneficial potential as anti-allergic, antibacterial, anticancer, anticlastogenic, anti-diarrhoeal, anti-fertility, anti-fungal, anti-hyperlipidemic, anti-hypertensive, antiinflammatory, anti-lieshmanial, anti-nociceptive, anti-oxidant, anti-viral, ascaricidal, cardioprotective, chemoprotective, diuretic, gastroprotective, hepatoprotective, hypothermic, neuropsychopharmacological and radioprotective agent. These actions and the lacunas in the specific areas have been discussed in this article. This will provide a platform for the future researchers especially in the less explored areas.

Ramirez *et al*, **2016** carried out the gastroprotective effect of quantified tannins (13.4%) from Syzygium cumini was determined. Gastric mucosal damage was induced in sixty eight rats by oral gavage administration of HCl/ethanol solution. For macroscopic and microscopic studies, 30 rats were divided into three groups consisting of a negative control, an Omeprazole group and a Tannins group. There was no significant difference in the number, size and surface area of macroscopic lesions between the three groups. Microscopic examination using Best's Ulcer Staging Index showed that Tannins had a very significant decrease in gastric mucosal damage with $p<0.01$. Average lymphocyte populations in the three groups showed no significant difference, although both the Tannins and Omeprazole group had fewer lymphocytes. Thirty-eight rats were studied for the amount of free radicals present after induction of gastric damage. A dose which consisted of 20.0 g

tannins/kg rat weight showed significantly lower stomach free radical concentrations. These findings suggest that tannins extracted from S. cumini have gastroprotective and anti-ulcerogenic effects.

G. C. Jagetia *et al*, **2015** had perform the effects of various concentrations (5, 10, 20, 30, 40, 50, 60, and 80 mg/kg body weight (b. wt.) of the leaf extracts of Syzygium cumini Linn. and Eugenia cumini (SCE, black plum, Jamun, family Myrtaceae) on the radiation-induced sickness and mortality in mice exposed to 10 Gy γ-irradiation were studied. The treatment of mice with different doses of SCE, consecutively for five days before irradiation, delayed the onset of mortality and reduced the symptoms of radiation sickness when compared with the nondrug-treated irradiated controls. All doses of SCE provide protection against the gastrointestinal death increasing the survival by 66.66% after treatment with 20, 30, and 40 mg/kg SCE versus a 12% survival in the irradiated control group (oil + irradiation). Similarly, SCE provided protection against the radiation-induced bone marrow death in mice treated with 10–60 mg/kg b.wt. of SCE. However, the best protection was obtained for 30 mg/kg b.wt. SCE, where the number of survivors after 30 days post-irradiation was highest (41.66%) when compared with the other doses of SCE.

K Karthic *et al***, 2016** reported the aqueous extract of *S. cumini* or *Eugenia jambolana* seeds and *Psidium guajava* leaves showed higher inhibition against the porcine pancreatic α-amylase among the medicinal plants studied. The α-amylase inhibitors from *S. cumini* seeds were separated from the extract by preparative thin layer chromatography into fractions with different R_f values. The fraction with Rf value between 0.285 and 0.43, which showed maximum inhibitory activity, was eluted and analyzed through LC-MS. The compounds identified from the seed extract of *S. cumini* were betulinic acid and 3, 5, 7, 4`-tetrahydroxy flavanone, which were reported earlier from *S. formosanum* and other plants. Dixon plot showed that the inhibition was noncompetitive in nature.

K. F. Migliato *et al***, 2016** reported a precise, accurate and low cost spectrophotometric method was developed and validated for routine determination of total polyphenols, as pyrogallic acid equivalents, from the percolated and lyophilized extract of *Syzygium cumini* (L.) Skeels fruits. Validation was assessed

experimentally and data were rigorously treated by statistical analysis. Analytical parameters were: linearity, interval (range), precision and recovery/accuracy, limit of detection (LOD, µg mL-1) and limit of quantification (LOQ, µg mL-1). The visible spectrophotometric method presented linearity (r2 = 0.9979 ± 0.0010) over the concentration range 0.25-7.5 µg mL-1 of standard pyrogallic acid, precision ≤ 2.918171%, recovery/accuracy ranging from 96.228693 to 107.17701%, LOD = 0.21 µg mL-1 and LOQ = 0.64 µg mL-1.

V Kumar *et al*, **2015** investigated plant mediated synthesis of metallic nanoparticles has been studied and reported, however, to date, the biomolecules involved in the synthesis of metallic nanoparticles have not been characterized. This study was therefore undertaken to characterize the biomolecules of *Syzygium cumini* involved in the synthesis of silver nanoparticles. Synthesis kinetics and morphological characterization of silver nanoparticles (SNP) synthesized using leaf extract (LE) and seed extract (SE) as well as their polar (water) fractions from Syzygium cumini were compared. The polyphenols content and high performance liquid chromatography (HPLC) profile of different fractions revealed good correlation between size and synthesis rate of SNP. SE contains more polyphenols and biochemical constituents than LE and therefore, showed higher synthesis rate and bigger sized SNP. To analyse the nature of biomolecules involved in the synthesis of SNP, LE and SE were fractionated on a polarity basis by solvent–solvent partitioning. Only the water fractions of LE and SE showed potential for SNP synthesis. Atomic force microscopy (AFM) and scanning electron microscopy (SEM) analysis of SNP indicated that all fractions catalyze the synthesis of spherical nanoparticles. The average size of SNP synthesized by LE, leaf water fraction, SE and seed water fraction were 30, 29, 92, and 73 nm respectively. Results suggest that only highly polar soluble constituents are responsible for SNP synthesis. The size of SNP was found to be directly correlated with the amount of polyphenols as well as surfactants present in the reaction solution. Thus, the amount of polyphenols could be one of the crucial parameters determining the size and distribution of SNP.

AIM & OBJECTIVE

AIM AND OBJECTIVES

Herbal research is still a vital source of novel pharmaceutical products moreover it is one of the best reservoirs for synthesis of novel structural based bioactive compounds. Near to 30% of the current using drugs comes from natural products and an additional 20% are structural alteration of the lead compounds from natural source. It is estimated that only 15% of higher plants have been investigated for potentially useful biological activity. In spite of the presence of known antioxidant medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease. Several traditional plant treatments like diabetes cardiac complicated disease, malaria, antiinsecticide etc. are used throughout the world. Herbal drugs and formulations are frequently considered to be less toxic and free from side effects than synthetic one. Hence, in modern days, huge attention has been directed towards recognition of plants with antidiabetic, cardiac complicated disease, malaria, antiinsecticide etc, ability that may be used effectively for human consumption. There has been rapid expansion of different classes of antioxidant drugs with distinctive pharmacological mechanism of action and, also, they have various toxicological profiles.

The literature review revealed that the extraction process with various solvents based on polarity with bark of *syzygium cuimini* has not been experimentally studied, as well as no detailed study has been carried out on the antioxidant as well as antimicrobial activity. From the literature survey it has been found that the barks of the selected plant (*syzygium cuimini*) are being used traditionally in the treatment of diabetes, based upon their traditional and ethnopharmacological information's.

The objective of the present research work focused on the investigation of phytochemical constituents of their various extractions based on their polarity and study of *in vitro* antioxidant as well as *in vitro* antimicrobial activity.

PLAN OF RESEARCH WORK

The following schedule of the research work has been planned and performed.

1. Selection of plants based upon literature survey and traditional claim.
2. Identification, authentication of plant(s) and collection of barks of the plant.
3. Preparation of extracts of various solvents based on polarity.
4. Investigation of preliminary phytochemical studies.
5. ***IN VITRO*** **STUDIES FOR VARIOUS EXTRACTS.**

5a. In vitro anti-microbial activity of various extracts.

1. Media preparation.
2. Carried out antimicrobial assay

   Well diffusion method

   Disc diffusion method.

5b. In vitro anti-oxidant activity of various extracts.

1. DPPH assay method.
2. Hydrogen peroxide scavenging assay method.
3. Nitric oxide scavenging assay method.

MATERIALS AND METHODS

MATERIALS AND METHODS

Plant collection:

The barks of *Syzygium Cumini* were collected from plants growing in the Krishnan koil, virudhunagar district during the months of July–August, 2016. It was then authenticated by Dr. Stephan, Dept. of Botany, The American College, Madurai.The bark was dried at 40^0 C for 15 days, then it was blended into coarse powder by electrical grinder. The powdered drug was passed through sieve No.22 to get uniform particle size

Materials

Petroleum ether, Hexane, Chloroform, Ethyl acetate, Methanol, Distilled water, Hydrogen peroxide, Griess reagent, DPPH (2, 2 -diphenyl-1-picrylhydrazyl), Hydrochloric acid, Sulphuric acid, Alpha-naphthol, Copper sulphate, Sodium hydroxide, Barfoed's solution, Benedict's solution, Potassium mercuric iodide, Potassium bismuth iodide, Iodine, Potassium iodide, Picric acid, Con.HNO_3, NH_4OH, Millon's reagent, Ninhydrin, Biuret reagent, Ammonia, 95% Ethanol, lead acetate, Potassium hydroxide, Phenolphthalein, Lead acetate, Ferric chloride, Agar-Agar, Potassium dihydrogen phosphate, Calcium carbonate, Rotary vaccum evaporator, UV Spectrophotometer, Aluminum foil, ordinary Filter paper, Whatmann filter paper,5000 ml RBF, Standard measuring flasks, Messaring cylinder, Beakers, Desiccators, Glass funnel, Test tubes, Holder, Glass rod.

EXTRACTION: [58]

Extraction procedure

Petroleum ether extraction

The barks (1000gm) were extracted by macerating with 2.5 litter of Petroleum ether for 7 days at room temperature in a dark cabinet. After 7 days, the extracts were collected by filtration, the marc was separated.Solvent extract was evaporated to dryness using rotary vacuum evaporator and weighed.

Hexane extraction

The residue of petroleum ether extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.5 litter of hexane for 7 days at room temperature in a dark cabinet. After 7 days, the extracts were collected by filtration, the marc was separated. Solvent extract was evaporated to dryness using rotary vacuum evaporator and weighed.

Chloroform extraction

The residue of hexane extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.5 litter of chloroform for 7 days at room temperature in a dark cabinet. After 7 days, the extracts were collected by filtration, the marc was separated.Solvent extract was evaporated to dryness using rotary vacuum evaporator and weighed.

Ethyl acetate extraction

The residue of chloroform extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.5 litter of ethyl acetate for 7 days at room temperature in a dark cabinet. After 7 days, the extracts were collected by filtration, the marc was separated.Solvent extract was evaporated to dryness using rotary vacuum evaporator and weighed.

Methanol extraction

The residue of ethyl acetate extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.5 liter of methanol for 7 days at room temperature in a dark cabinet. After 7 days, the extracts were collected by filtration, the marc was separated.Solvent extract was evaporated to dryness using rotary vacuum evaporator and weighed.

Distilled water extraction

The residue of methanol extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.5 liter of distilled water for 7 days at room temperature in a dark cabinet. After 7 days, the extracts were collected by filtration, the marc was separated.Solvent extract was evaporated to dryness using rotary vacuum evaporator and weighed.

PHYSICAL EVALUATION [52-57]

Determination of total ash:

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both “Physiological ash” which is derived from the plant tissues itself and “Non physiological” which is the residue of the extraneous matter (e.g.,) sand and soil adhering to the plant surface

Procedure:

Weigh accurately 1 g of the air-dried drug in a tared platinum or silica dish and incinerate at a temperature not exceeding 450º until free from carbon, cool and weigh. If a carbon-free ash is not obtained, wash the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper until the ash is white or nearly white, add the filtrate to the dish, evaporate to dryness and ignite at a temperature not exceeding 450º. Calculate the percentage of ash on the dried drug basis. The procedure was repeated thrice and average was calculated and noted in table I

Determination of acid insoluble ash:

This method is designed to measure the amount of silica present, especially as sand and siliceous earth

Procedure:

The total ash was taken in a silica crucible with 25 ml of 2M hydrochloric acid for 5 minutes, collect the insoluble matter in a Gooch crucible or on an ashless filter paper, wash with hot water, ignite, cool in a desiccator and weigh. Calculate the percentage of acid-insoluble ash on the dried drug basis. The procedure was repeated thrice and average was calculated and noted in table I

Determination of water insoluble ash:

Procedure:

The total ash was taken in a silica crucible with 25 ml of water for 5 minutes, collect the insoluble matter in a Gooch crucible or on an ashless filter paper, wash

with hot water, ignite, cool in a desiccator and weigh. Calculate the percentage of acid-insoluble ash on the dried drug basis. The procedure was repeated thrice and average was calculated and noted in table I

Determination of Loss on drying

About 2gm of ground air dried material was taken in a previously dried china dish, this was placed in a hot air oven and heated at 100^{o}C for 1 hour. Then it was cooled to room temperature, weighed. The percentage moisture content was calculated with reference to air dried drug material. The procedure was repeated thrice and average was calculated and noted in table I

Then a small fraction of all the extracts were subjected to various chemical tests for the identification of various plant constituents as in the procedure given below and the findings are reported in table. III.

INVESTIGATION OF PRELIMINARY PHYTOCHEMICAL STUDIES: [52-53]

The various extracts of *Syzygium cumini* obtained were subjected to qualitative analysis to test the presence of various phytochemical constituents like alkaloids, carbohydrates, glycosides, flavonoids, steroids, aminoacid, phenols, proteins, tannins etc.

Procedure:

1. TESTS FOR CARBOHYDRATES:

A small portion of the extract was dissolved separately in 5ml of water and filtered. The filtrate was subjected to the following tests.

a. Molish's Test:

To 2-3 ml of the extract add few drops of alpha-naphthol in alcohol followed by conc. H_2SO_4 was added through the sides of the test tube violet colour ring is formed at the junction of two liquids shows presence of carbohydrates.

b. Fehling's solution:

To a solution of the substance, a mixture of equal parts of Fehling's solution A and B was added and the test tube was heated on a water bath. Red colour precipitate shows presence of carbohydrates.

c. Barfoed's test:

To a small portion of the substance, Barfoed's solution was added and it was boiled. Red colour precipitate shows presence of carbohydrates.

d. Benedict's test:

To a small portion of the substance, Benedict's solution was added and mixed well and it was boiled. Then it was allowed to cool. Red colour solution shows presence of carbohydrates.

2. TESTS FOR ALKALOIDS:

The small portions of the extracts were dissolved in suitable solvent and each extract was stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate was tested for alkaloids by using the following reagents.

a. Mayer's test.

To 2-3 ml filterate, add few drops Mayer's reagent (potassium mercuric iodide) which gives white precipitate shows presence of alkaloids.

b. Dragendroff's test

To 2-3 ml filterate, add few drops Dragendroff's reagent (potassium bismuth iodide) which gives orange brown precipitate shows presence of alkaloids.

c. Wagner's test

To 2-3 ml filterate, add few drops Wagner's reagent (iodine+potassium iodide) which gives reddish brown precipitate shows presence of alkaloids.

d. Hager's test

To 2-3 ml filterate, add few drops Hager'sreagent (saturated picric acid) which gives yellow colour precipitate shows presence of alkaloids.

e. Muroxide test

To 2-3 ml filterate, add few drops Con.HNO_3 evaporate to dryness. Cool and add 2 drops of NH_4OH which gives purple colour shows presence of alkaloids.

3. TESTS FOR PROTEINS AND AMINO ACIDS:

a. Millon's Test:

To 3ml extract was added with Millon's reagent and it was boiled which gives white precipitate shows presence of aminoacids.

b. Ninhydrin test:

To 3ml extract was added with 5% Ninhydrin solution and it was boiled. Then it was allowed to cool which gives bluish colour shows presence of aminoacids.

c. Biuret Test:

To 3ml extract was added with Biuret reagent and violet colour was shows presence of protein.

d. Xanthoprotein Test:

To 3ml extract, concentrated nitric acid was added and the white precipitate was shows presence of protein.

4. TESTS FOR SAPONINS:

a) Foam Test:

1 ml of the test solution was taken in a measuring cylinder. To this, 20 ml of distilled water was added and shaked well which gives persistent foam shows presence of saponins.

b) Haemolysis Test:

The extracts of the plant was spread over a glass slide to form a thin film layer on which a drop of human blood was placed and spread over the extract layer. After 30 minutes, the slide was examined under microscope for change in the structure and shape of red blood cells. Control was always maintained to see the change in red blood cells structure for haemolysis

4. TEST FOR GLYCOSIDES:

a. Baljet' s Test:

To 3ml extract added sodium picrate solution which gives orange colour was shows presence of glycosides.

b. Legal's Test:

To 3ml extract, few ml of pyridine, 2 drops of nitroprusside and a drop of 20% NaOH solution were added which gives pink colour shows presence of saponins.

c. Borntrager's Test:

To 3ml extract was mixed with dilute.H_2So_4 and filtered. The filtrate was shaken with chloroform and the chloroform layer was separated. To this dilute ammonia was added which ammoniacal layer turns pink colour.

5. TEST FOR FLAVONOIDS:

a. Shinoda test

To dry powder add 5 ml 95% ethanol, Con.Hcl and 0.5gm magnesium turnings which gives pink colour shows presence of flavonoids.

b. To 2 ml of residue add lead acetate solution which gives yellow colour precipitate shows presence of flavonoids.

6. TESTS FOR GUMS AND MUCILAGES:

a. To a small amount of extract, 25ml of absolute alcohol was added and then it was filtered. The precipitate was examined for its swelling properties.

b. To the extract, ruthenium red solution was added.

7. TESTS FOR FIXED OILS AND FATS:

a. Spot Test:

Extracts were taken and they were pressed between filter paper and the paper was noted.

b. Few drops of 0.5N alcoholic potassium hydroxide were added to various extracts with few drops of phenolphthalein. The mixture was heated on a water bath for 1-2 hours.

8. TEST FOR PHYTOSTEROLS:

The extracts were refluxed separately with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted with distilled water and extracted with ether. The etherial extract was evaporated and the residue was subjected to Liebermann- Burchard test.

a. Liebermann-burchard test:

Extracts were shaken with few drops of dry acetic acid. To this, 3ml of acetic anhydride was added followed by 3 drops of conc. sulphuric acid which gives green colour shows presence of phytosterols.

b. Salkowski reactions

To 2 ml of extract add 2 ml of chloroform and 2 ml of Con.H_2SO_4 which gives organic layer appear red colour shows presence of phytosterols.

9. TEST FOR PHENOLIC COMPOUNDS AND TANNINS:

Small quantities of alcoholic and aqueous extracts add few drops of following reagents

a. 5%$Fecl_3$ solution

Deep blue colour shows presence of tannins.

b. 10% Lead acetate solution

White colour precipitate was shows presence of tannins.

c. Gelatin solution:

White colour precipitate was shows presence of tannins.

d. Dil.HNO_3 solution:

Reddish to yellow colour shows presence of tannins.

***IN VITRO* ANTIMICROBIAL STUDIES:**

a) Well diffusion method

b) Disc diffusion method

1. Media preparation

Bacterial medium (Muller Hinton Agar)

36 g of Muller Hinton Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15 lb pressure for 15 minutes. The sterilized media were poured into petri dishes. The solidified plates were pored with 6 mm dia. cork borer. The plates with wells were used for the antibacterial studies.

Test against standard controls

Commercially available antibiotic disc Ciprofloxacin (10 µg) and was used as standard control for the entire test microorganism.

Bacterial inoculums

Bacterial inoculums were prepared by inoculating a loopful of test organisms in 5 ml nutrient broth and incubated at 37ºC for 5 to 8hrs till a moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standard (WHO drug information, 1993) and the culture was diluted with sterile distilled water if necessary which corresponds to the cell density of 1.5 x 108 (cfu/ml).

2. Antimicrobial assay

WELL DIFFUSION METHOD[20]

Antibacterial of the plant extract was tested using well diffusion method (Bauer et al., 1996). The prepared culture plates were inoculated with different selected strains of bacteria using streak plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were loaded into the well using sterile syringe. The plates were incubated 24 hours at 37 ± 2ºC bacterial plates. The plates were observed for inhibition zone formation around the well. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The various extract of *Syzygium cumini* bark was used throughout the study. The extracts of 25, 50,100µg/0.1 ml were tested against 4 different bacterial pathogens such as Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli for their antimicrobial activity. It was demonstrated by well diffusion assay and reported in table III.

DISC DIFFUSION METHOD[59]

Principle

Paper discs impregnated with specific antibiotics or the test substances are placed on the surface of the Muller Hinton agar medium inoculated with the target organisms, which is recommended for the diffusion of antimicrobial agents as

described in NCCLS approved standard. The plates are incubated and the zones of inhibition around each disc are measured.

Procedure

Petri dishes were prepared with a base layer of Mueller Hinton agar. At twenty-four hours culture of selected bacteria like Bacillus subtilis, , Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli were mixed with physiological saline solution and the turbidity was corrected by adding sterile physiological saline until a McFarland turbidity standard of 0.5 (108 CFU/mL). Afterwards, a top layer of Mueller Hinton agar inoculated with 0.2% microbial suspension was poured over the Petri dishes. Sterile filter discs (6 mm in diameter) were impregnated with 16 µL of plant extract (25, 50,100 µg/0.1mL) dissolved in DMSO and placed on the inoculated plates. Ciprofloxacin was used as control. The plates were incubated at 35ºC for 18 hours Microbial growth inhibition was determined as the diameter of the inhibition zones around the discs and reported in table IV.

***IN VITRO* ANTIOXIDANT STUDIES[23-27]**

DPPH (2, 2 -diphenyl-1-picrylhydrazyl) Assay:-

Principle:

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as

(DPPH) + (H-A) $\longrightarrow$ DPPH-H + (A)

(Purple) (Yellow)

Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPH -H and as consequence the absorbance's decreased from the DPPH radical to the DPPH -H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Reagents:

1. Methanolic solution of DPPH (0.1 mM): 39.4 mg of DPPH was dissolved in one liter of analytical grade methanol.

2. Ascorbic acid

Procedure:

To 1.0 ml of DPPH (0.1 mM) solution was added to 3.0 ml of bark various extract solution in methanol at different concentration (100-300 µg/ml) and allowed to react in dark at room temperature for 30 min. Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (100 to 300 µg/ml) was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. and reported in table V& chart I.II.III.IV

The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = \frac{(A_0 - A_1) \times 100}{A_0}$$

A_0 - Absorbance of control

A_1 - Absorbance in the presence of plant extract

HYDROGEN PEROXIDE SCAVENGING ACTIVITY

Reagents

1. Phosphate buffer (0.1M, pH 7.4)

2. H_2O_2 (2mM) in phosphate buffer.

Procedure

A solution of H_2O_2 (2mM) was prepared in phosphate buffer. Bark extracts at the concentration (50 - 250µg/ml) were added to H_2O_2 solution (0.6ml) and the total

volume was made up to 3ml. The absorbance of the reaction mixture was recorded at 230nm in a spectrophotometer (Shimadzu, UV-1700 Pharmaspec). A blank solution containing phosphate buffer, without H_2O_2 was prepared and reported in table VI & chart V, VI, VII, VIII.

The extent of H_2O_2 scavenging of the plant extracts was calculated as

$$\% \text{ scavenging of hydrogen peroxide} = \frac{(A_0 - A_1) \times 100}{A_0}$$

A_0 - Absorbance of control.

A_1 - Absorbance in the presence of plant extract.

NITRIC OXIDE SCAVENGING ACTIVITY

Principle

Sodium nitroprusside in Phosphate buffered saline (PBS), at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that are estimated spectrophotometrically at 546nm.

Reagents

1. Sodium nitroprusside (10mM)

2. Phosphate buffered saline (pH 7.4)

3. Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthylethylene diamine dihydrochloride)

Procedure

sodium nitroprusside (10mM) in phosphate buffered saline was mixed with different concentrations (200-450µg/ml) of methanol extract of each plant were dissolved in methanol and incubated at 30°C for 2 hours. The same reaction mixture without the extract but the equivalent amount of ethanol served as the control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H_3P0_4 and

0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride was immediately read at 550 nm. Inhibition of nitrite formation by the plant extracts and the standard antioxidant ascorbic acid were calculated relative to the control. Inhibition data (percentage inhibition) were linearized against the concentrations of each extract and standard antioxidant and reported in table VII & chart IX, X, XI, and XII.

$$\% \text{ scavenging of nitric oxide} = \frac{(A_0 - A_1) \times 100}{A_0}$$

A_0 - Absorbance of control.

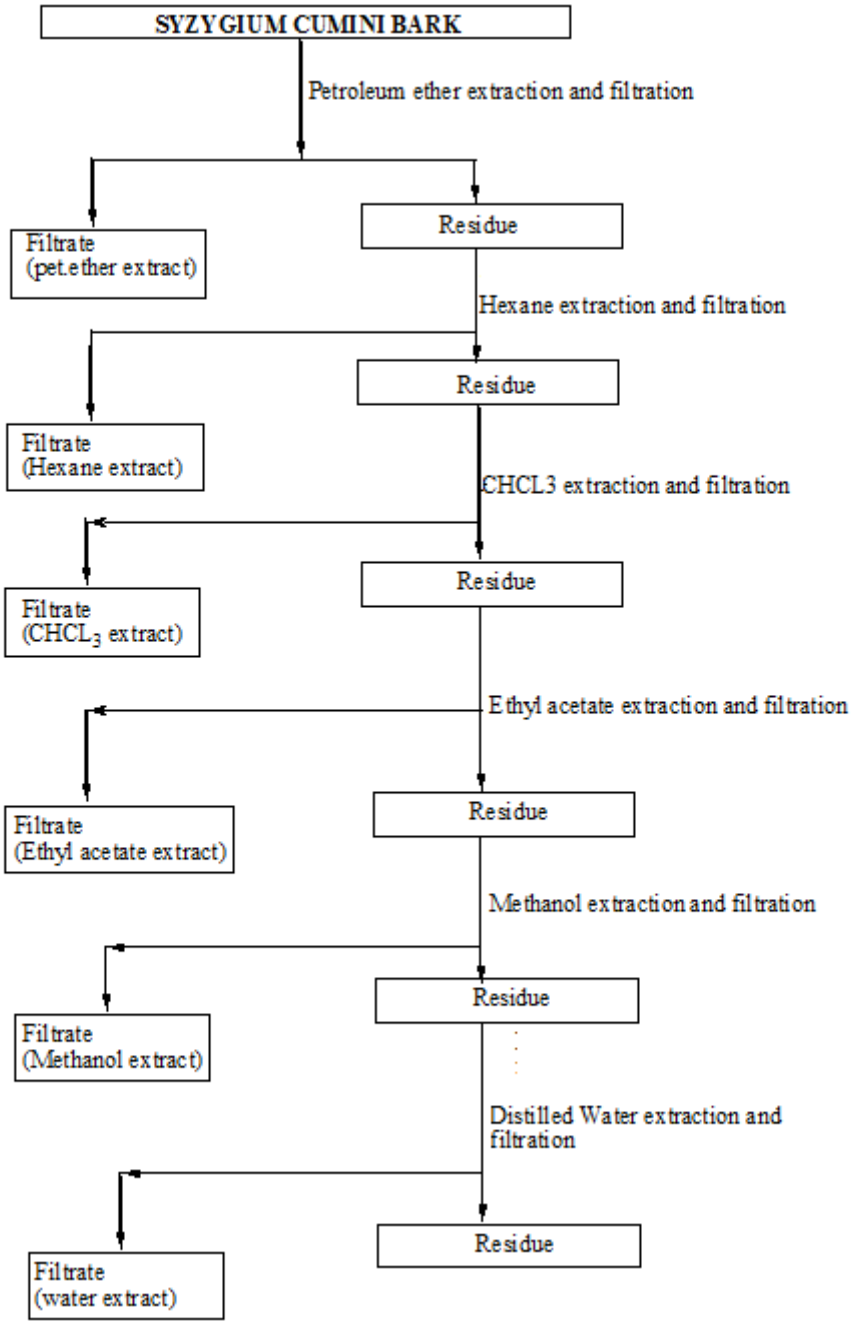
A_1 - Absorbance in the presence of plant extract.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The protocol of various extracts has been summarized as **scheme I.** based on the scheme the powdered drug (barks of the *Syzygium cumini*) has extracted by cold percolation method with sufficient solvents based on their polarity (Pet. ether, $CHCl_3$, Ethyl acetate, Methanol and water). The obtained extracts allowed concentrating by rotary vacuum evaporator. The concentrated extracts subjected to physiochemical evaluation, as well as investigation of preliminary phytochemical studies. The results of the physiochemical evaluation and phytochemical constituents shown as **Table I & II**. From the tables, water insoluble and acid insoluble materials are being about 8.5% and 13.5% respectively. Moreover pet. ether extracts give positive results of steroids and fats & oils. Hexane extract give positive results for proteins, glycosides, and alkaloids, $CHCl_3$ extract give positive results for steroids, alkaloids, tannins and flavonoids also. Ethyl acetate extract gives positive results for alkaloids and tannins, Methanol extract gives positive results for amino acid, flavonoids and tannins and the aqueous extract contains proteins, flavonoids and tannins.

Scheme: 1



General Scheme of extraction protocol

TABLE I

PHYSIOCHEMICAL EVALUATION

Physiochemical	Experiments			
	I (% w/w)	II (% w/w)	III (% w/w)	Average (% w/w)
Total ash	24%	26%	25%	25%
Water insoluble ash	8.5%	8.6%	8.4%	8.5%
Acid insoluble ash	13.4%	13.6%	13.5%	13.5%
Loss on drying	15.92%	15.91%	15.92%	15.92%

Table II

PHYTOCHEMICAL SCREENING ON VARIOUS EXTRACTS OF

SYZYGIUM CUMINI (L.).

S.NO	EXTRACTION	CHEMICAL CONSTIUENTS									
		Car	Pro	AA	Ster	Gly	Alk	Tan	F&O	Fla	Vol
1.	Pet. ether	-	-	-	+	-	-	-	+	-	-
2.	Hexane	-	+	-	-	+	+	-	-	-	-
3	Chloroform	-	-	-	+	-	+	+	-	+	-
4	Ethylacetate	-	-	-	-	-	+	+	-	-	-
5	Methanol	-	-	+	-	-	-	+	-	+	-
6.	Aqueous	+	+	-	-	-	+	-	-	+	-

Car-Carbohydrate, Pro-Protein, AA-Aminoacid, Ster-Steroid, Alk-Alkaloid, Tan-Tannins, Fla-Flavanoid, F&O-Fat &Oils,Vol-Volatile oils

+ = indicates the presence of constituents

- = indicates the absence of constituents

Table III

WELL DIFFUSION METHOD

S. No.	Strains	Zone of inhibition (mm)												
		Extracts (µg/0.1ml)												
		Petroleum ether			Chloroform			Ethyl acetate			Methanol			Std
		25	50	100	25	50	100	25	50	100	25	50	100	10
1.	***E. coli***	-	1	2	-	-	-	-	1	2	-	-	1	10
2.	***P.aeruginosa***	-	-	-	-	-	-	-	-	-	-	1	2	7
3.	***S.aureus***	-	1	3	1	2	4	-	-	-	1	2	3	10
4.	***B.substilus***	3	4	5	2	3	4	-	1	2	1	2	3	11

E.coli*-Escherichia coli*

P.aeruginosa-*Pseudomonas aeruginosa*

S.aureus-*Staphylococcus aureus*

B.substilus-*Bacillus subtilis*

TABLE IV

DISC DIFFUSION METHOD

S.No	Strains	Zone of inhibition (mm)											
		Extracts (µg/0.1ml)											
		Peterolum ether			Chloroform			Ethyl acetate			Methanol		Std
		25	50	100	25	50	100	25	50	100	25	50	100	10
1.	***E.coli***	-	2	4	-	-	-	-	1	3	-	-	2	11
2.	***P.aeruginosa***	-	-	-	-	-	-	-	-	-	-	2	3	8
3.	***S.aureus***	-	2	3	1	3	4	-	-	-	1	2	3	10
4.	***B.substilus***	3	4	6	3	4	5	-	1	2	2	3	4	11

E.coli*-Escherichia coli*

P.aeruginosa-*Pseudomonas aeruginosa*

S.aureus-*Staphylococcus aureus*

B.substilus-*Bacillus subtilis*

TABLE V

DPPH RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS ($CHCl_3$, ETHYL ACETATE, METHANOL) AGAINST STANDARD (ASCORBIC ACID)

S.No	Conc. (µg/ml)	Standard		Chloroform		Ethyl acetate		Methanol	
		Absorbance (Mean±SEM)	% Inhibition	Absorbance (Mean±SEM)	% inhibition	Absorbance (Mean±SEM)	% inhibition	Absorbance (Mean±SEM)	% inhibition
1	20	1.059 ±0.0017	88.28	1.342 ±0.0016	32.15	1.359 ±0.0017	29.15	1.265 ±0.0015	21.58
2	40	0.809 ±0.0018	96.52	1.166 ±0.0019	48.71	1.199 ±0.0020	39.36	1.106 ±0.0017	36.62
3	60	0.685 ±0.0024	109.25	0.957 ±0.0021	56.16	0.997 ±0.0023	52.78	0.974 ±0.0021	58.13
4	80	0.469 ±0.0027	126.72	0.803 ±0.0024	63.15	0.846 ±0.0026	59.12	0.823 ±0.0025	72.52
5	100	0.273 ±0.0029	131.05	0.643 ±0.0028	70.65	0.683 ±0.0029	64.69	0.730 ±0.0028	81.34
6	250	0.243 ±0.0031	154.05	0.592 ±0.0030	78.32	0.637 ±0.0031	75.06	0.656 ±0.0032	89.75
7	300	0.239 ±0.0033	162.34	0.453 ±0.0034	86.19	0.542 ±0.0035	97.58	0.582 ±0.0036	97.86
IC_{50} values		**IC_{50} =6.1 µg/ml**		**IC_{50} =41 µg/ml**		**IC_{50} =48 µg/ml**		**IC_{50} =45 µg/ml**	

TABLE VI

HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF VARIOUS EXTRACTS ($CHCl_3$, ETHYL ACETATE, METHANOL) AGAINST STANDARD (ASCORBIC ACID)

S. No	Conc. (µg/ml)	Standard		Chloroform		Ethyl acetate		Methanol	
		Absorbance Mean±SEM	% Inhibition	Absorbance Mean±SEM	% inhibition	Absorbance Mean±SEM	% inhibition	Absorbance Mean±SEM	% Inhibition
1	20	1.059 ±0.0017	89.58	1.490 ±0.0018	36.58	1.371 ±0.0016	27.95	1.377 ±0.0017	28.54
2	40	0.809 ±0.0018	98.35	1.283 ±0.0020	59.78	1.201 ±0.0019	42.56	1.214 ±0.0020	46.82
3	60	0.785 ±0.0021	112.01	1.087 ±0.0023	68.05	1.056 ±0.0021	53.69	1.071 ±0.0023	59.51
4	80	0.682 ±0.0024	124.73	0.901 ±0.0027	76.25	0.892 ±0.0025	61.78	0.925 ±0.0026	69.42
5	100	0.563 ±0.0027	132.24	0.842 ±0.0030	82.32	0.806 ±0.0029	66.56	0.830 ±0.0029	75.36
6	250	0.421 ±0.0030	148.62	0.765 ±0.0032	88.09	0.756 ±0.0031	78.92	0.732 ±0.0032	83.78
7	300	0.395 ±0.0034	165.36	0.623 ±0.0036	94.61	0.683 ±0.0033	94.36	0.612 ±0.0036	92.75
IC_{50} Values		**IC_{50} =5.5 µg/ml**		**IC_{50} =31 µg/ml**		**IC_{50} =46 µg/ml**		**IC_{50} =40 µg/ml**	

TABLE VII

NITRIC OXIDE SCAVENGING ACTIVITY OF VARIOUS EXTRACTS ($CHCl_3$, ETHYL ACETATE, METHANOL) AGAINST STANDARD (ASCORBIC ACID)

S. No	Conc. (µg/ml)	Standard		Chloroform		Ethyl acetate		Methanol	
		Absorbance Mean±SEM	% Inhibition	Absorbance Mean±SEM	% inhibition	Absorbance Mean±SEM	% inhibition	Absorbance Mean±SEM	% Inhibition
1	20	1.063 ±0.0017	87.26	1.160 ±0.0015	41.32	1.192 ±0.0014	21.52	1.120±0.0012	32.96
2	40	0.819 ±0.0018	96.37	1.118 ±0.0021	61.01	1.143 ±0.0017	36.56	1.014 ±0.0015	50.51
3	60	0.735 ±0.0021	110.01	1.005 ±0.0024	69.74	1.017 ±0.0018	52.74	0.913 ±0.0018	68.52
4	80	0.698 ±0.0024	128.23	0.921 ±0.0027	78.05	0.939 ±0.0020	60.04	0.839 ±0.0021	73.02
5	100	0.525 ±0.0027	133.54	0.855 ±0.0029	83.12	0.878 ±0.0024	67.58	0.755 ±0.0024	78.51
6	250	0.465 ±0.0030	153.73	0.739 ±0.0032	89.45	0.754 ±0.0028	77.45	0.721 ±0.0029	84.23
7	300	0.336 ±0.0034	165.78	0.613 ±0.0036	93.78	0.624 ±0.0031	93.71	0.654±0.0034	96.27
IC_{50} Values		**IC_{50} =6.8 µg/ml**		**IC_{50} =38 µg/ml**		**IC_{50} =46 µg/ml**		**IC_{50} =39 µg/ml**	

***In Vitro* antimicrobial assay:**

The obtained bark extracts of *Syzygium cumini (L)* were subjected to antimicrobial assay by well diffusion method and disc diffusion method, the results were posted as table III, IV. From the tables, four extracts (pet. ether, $CHCl_3$, Ethyl acetate, Methanol) of the titled plant have shows significant activity against gram + ve as well as gram - ve bacteria. Among them pet. ether extract of *Syzygium cumini (L)* has shows 3mm of zone of inhibitory activity about 25µg/0.1ML as shows significant activity against *B. Substilus* in well diffusion method and 3mm of zone of inhibitory activity about 25µg/0.1ML as shows significant activity against *B. Substilus* in disc diffusion method. Ciprofloxacin 10µg/0.1ML used as standard drug for both methods.

***In Vitro* antioxidant assay:**

DPPH assay;

The free radical scavenging activity of *syzygium cumini (L.)* bark extracts of ($CHCl_3$, EA and MeOH) was studied by its ability to reduce the DPPH a stable free radical. DPPH is nitrogen centered stable free radical having maximum absorbance at 517 nm in alcoholic solution. It becomes a stable diamagnetic molecule on accepting an electron or hydrogen atom. In the presence of an extract capable of donating a hydrogen atom, the free radical nature of DPPH is lost and the purple colour change to yellow (diphehylpicrylhydrazine). The DPPH inhibition of bark extracts of ($CHCl_3$, EA and MeOH) are shown in Fig V. the results are shown as the relative activities against the STD ascorbic acid.

In DPPH assay $CHCl_3$ extract of titled plant has shown as maximum scavenging activity the absorbance range from 1.342 ± 0.0016 to 0.643 ± 0.0028 (mean ± SEM) while compare with other two extracts (EA and MeOH) absorbance ranging from 1.359 ± 0.0017 (mean ± SEM) to 0.637 ± 0.0031 and 1.265 ± 0.0015 to 0.582 ± 0.0036 respectively. All the values of absorbance taken as mean of triplicates. The half inhibitory concentration (IC_{50}) of various extracts of $CHCl_3$, EA and MeOH about 41µg/ML, 48 µg/ML and 45 µg/ML respectively.

H_2O_2 Scavenging activity:

H_2O_2 scavenging activity of the various extracts of the *syzygium cumini (L.)* may due to free radical mediated deoxyribose damage was assessed by means of the hydroxyl radical scavenging assay the Fenton reaction generates OH^* radicals which degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component and may cause to DNA fragmentation and DNA strand breakage. The H_2O_2 inhibition of bark extracts of ($CHCl_3$, EA and MeOH) are shown in Fig V. the results are shown as the relative activities against the STD ascorbic acid.

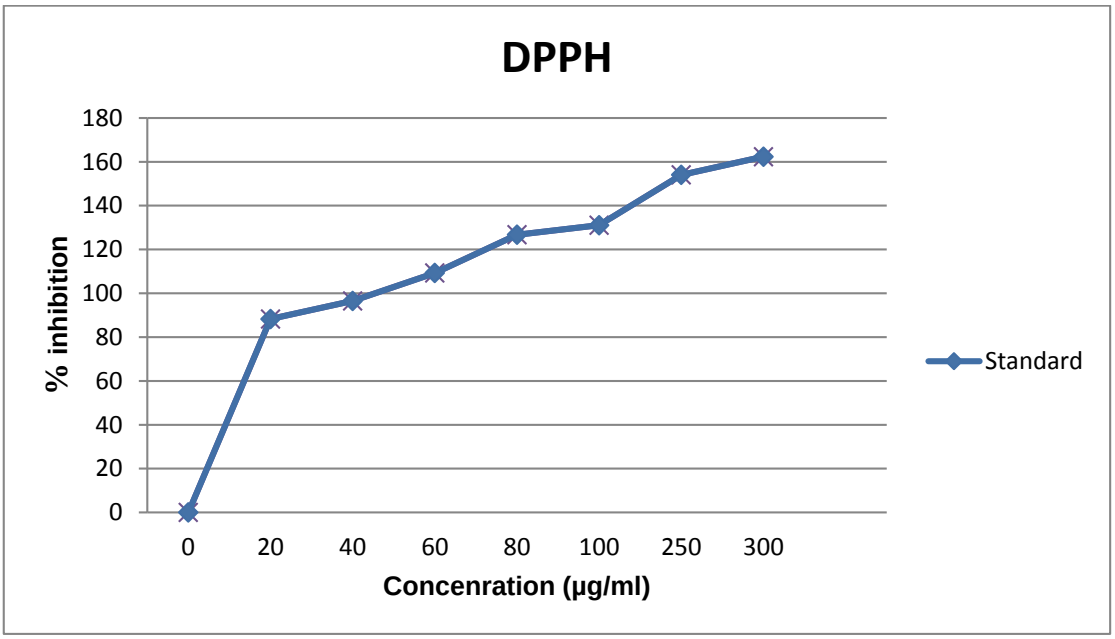
In H_2O_2 scavenging activity of the $CHCl_3$ extract of the titled plant has shown significance scavenging activity, the absorbance ranging from 1.490±0.0018 to 0.623±0.0036 (mean ± SEM) while compare with other two extracts (EA and MeOH) absorbance ranging from 1.371±0.0016 (mean ± SEM) to 0.683 ±0.0033 (mean ± SEM) and 1.377 ±0.0017 (mean ± SEM) to 0.612±0.0036 (mean ± SEM) respectively. All the values of absorbance taken as mean of triplicates. The half inhibitory concentration (IC_{50}) of various extracts of $CHCl_3$, EA and MeOH about 31µg/ML, 46 µg/ML and 40 µg/ML respectively.

Nitric oxide (NO) Scavenging activity:

Nitric oxide (NO), being a potent pleiotropic mediator in physiological processes and a diffusible free radical in the pathological conditions, reacts with superoxide anion and form a potentially cytotoxic molecule, the 'peroxynitrite ($ONOO^-$)'. Its protonated form, peroxynitrous acid (ONOOH), is a very strong oxidant. The main route of damage is the nitration or hydroxylation of aromatic compounds, particularly tyrosine. Under physiological conditions, peroxynitrite also forms an adduct with carbon dioxide dissolved in body fluid and responsible for oxidative damage of proteins in living systems. The NO inhibition of bark extracts of ($CHCl_3$, EA and MeOH) are shown in Fig XV. the results are shown as the relative activities against the STD ascorbic acid.

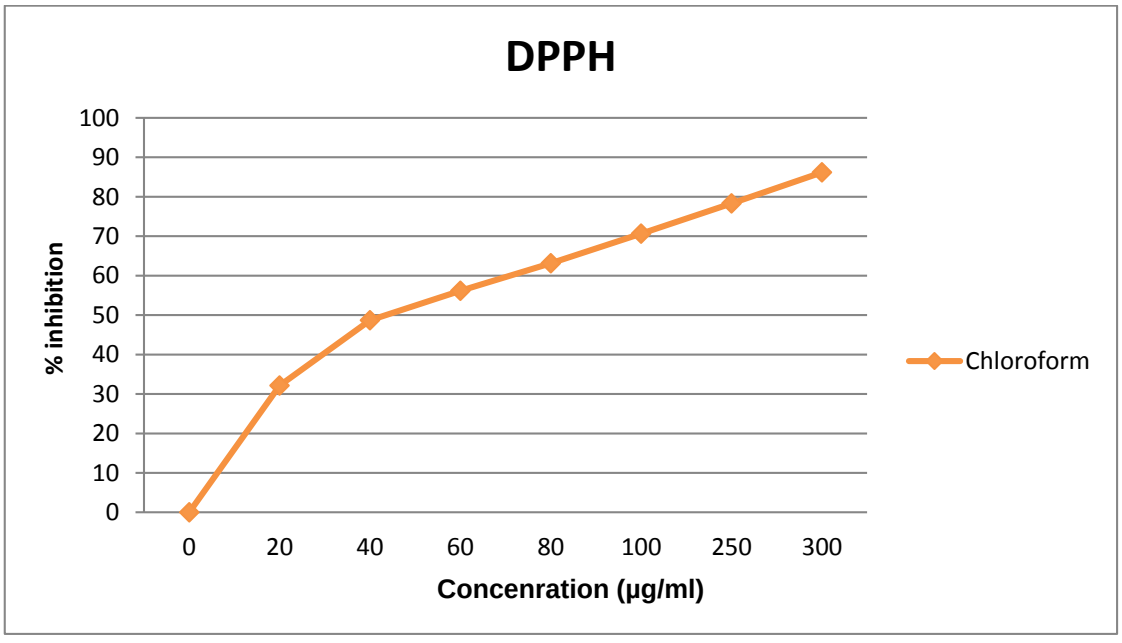
NO scavenging activity of the $CHCl_3$ extract of the titled plant has shown significance scavenging activity, the absorbance ranging from 1.160±0.0015 to 0.613±0.0036 (mean ± SEM) while compare with other two extracts (EA and MeOH) absorbance ranging from 1.192±0.0014 (mean ± SEM) to 0.624±0.0031 (mean ± SEM) and 1.120±0.0012 (mean ± SEM) 0.654±0.0034 (mean ± SEM) respectively. All the values of absorbance taken as mean of triplicates. The half inhibitory concentration (IC_{50}) of various extracts of $CHCl_3$, EA and MeOH about 38 µg/ML, 46 µg/ML and 39 µg/ML respectively.

FIGURE I : DPPH RADICAL SCAVENGING ACTIVITY OF STANDARD (ASCORBIC ACID)



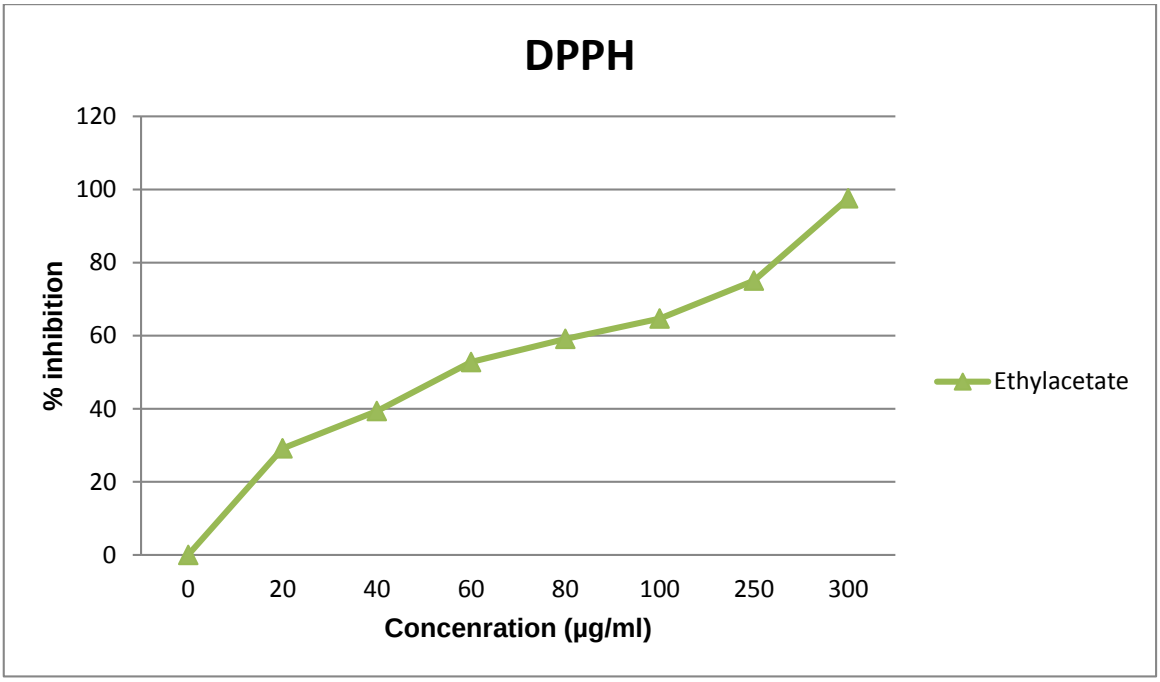
Standard-Ascorbic acid IC_{50}-6.1 µg/ml

FIGURE II: DPPH RADICAL SCAVENGING ACTIVITY OF $CHCl_3$ EXTRACT



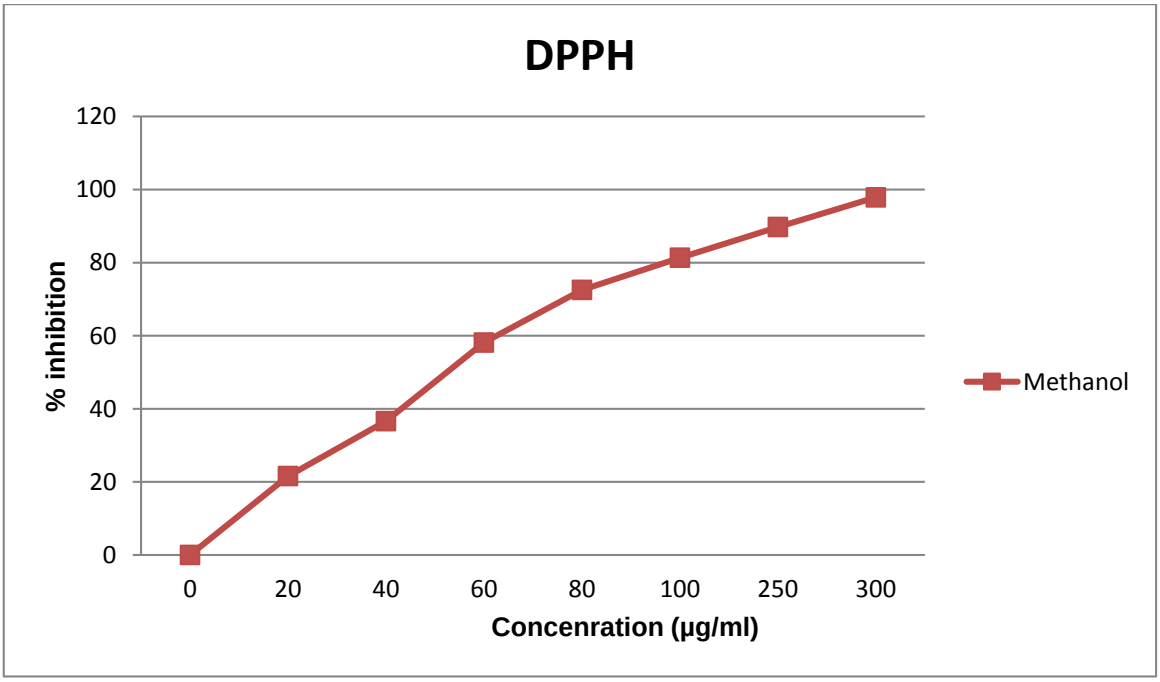
IC_{50} =41 µg/ml

FIGURE III: DPPH RADICAL SCAVENGING ACTIVITY OF ETHYL ACETATE EXTRACT



IC_{50} =48 µg/ml

FIGURE IV : DPPH RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACT



IC_{50} =45 µg/ml

FIGURE V: DPPH RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS ($CHCl_3$, ETHYL ACETATE, METHANOL) AGAINST STANDARD (ASCORBIC ACID)

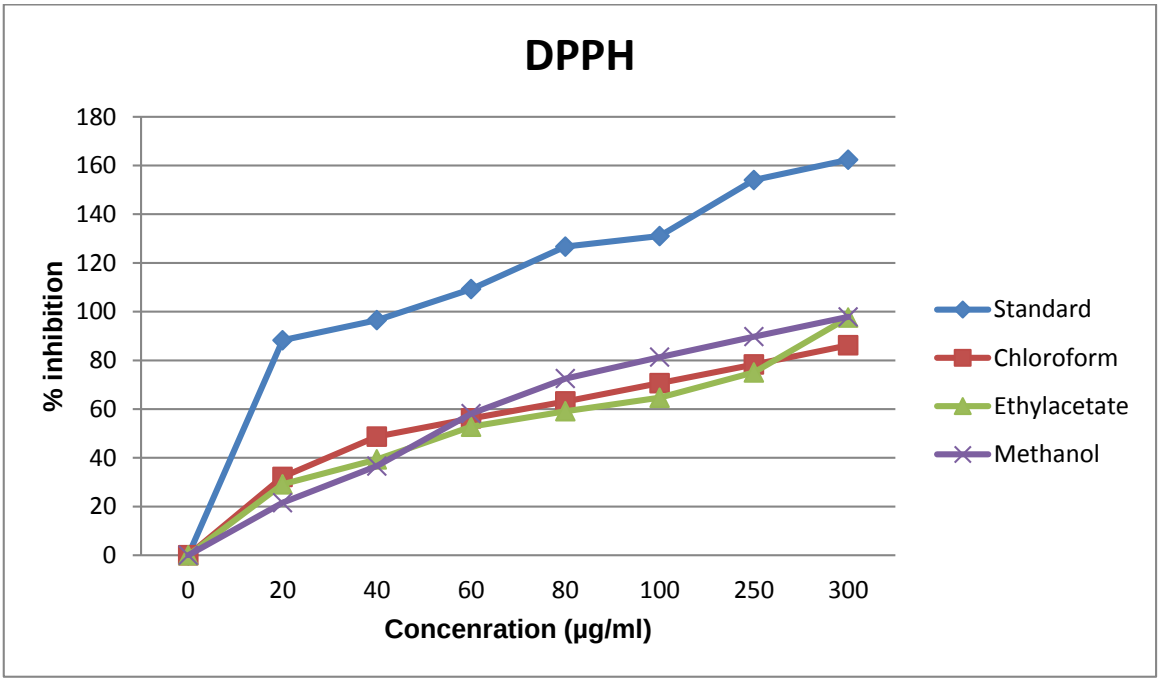
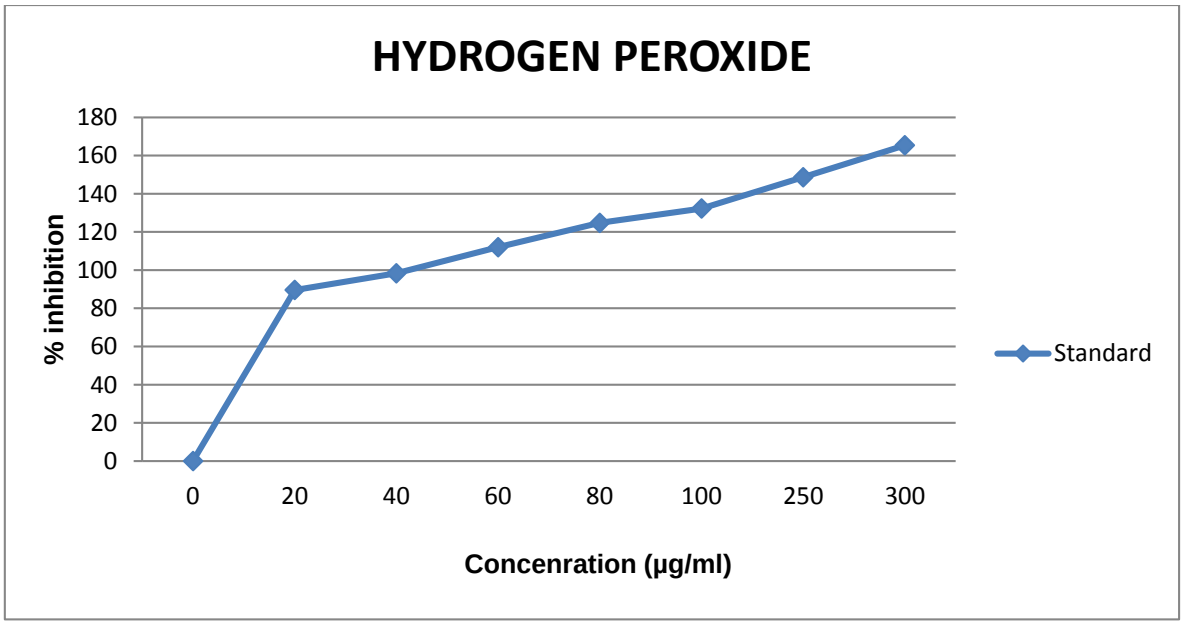
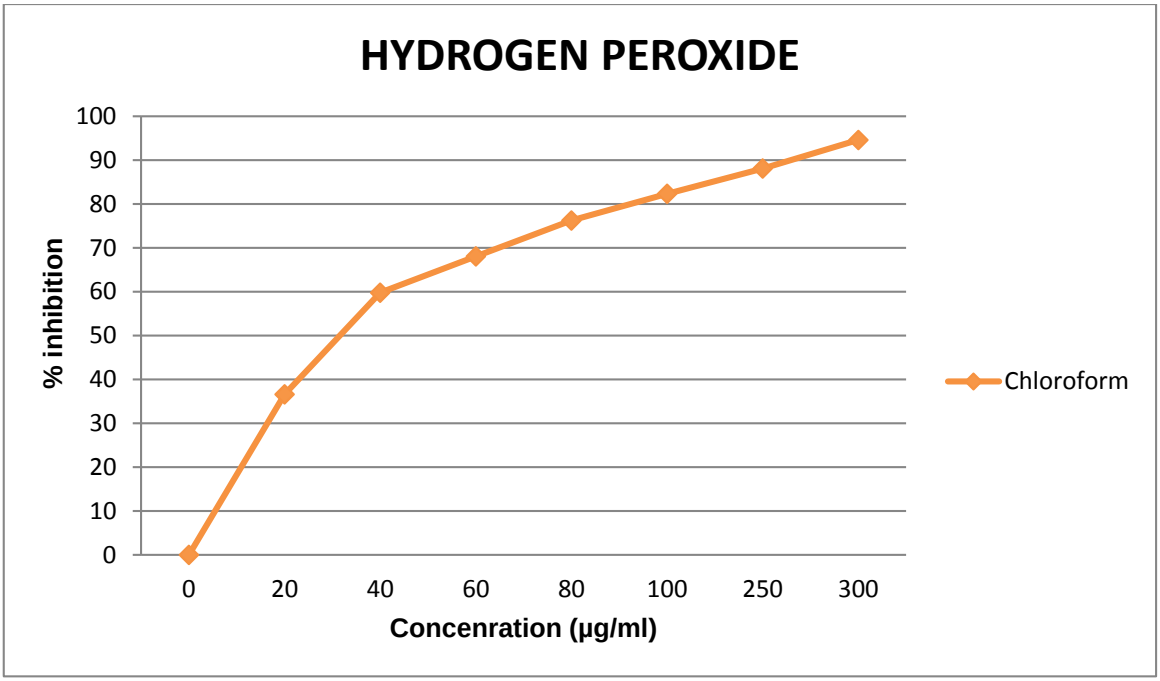


FIGURE VI : HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF STANDARD (ASCORBIC ACID)



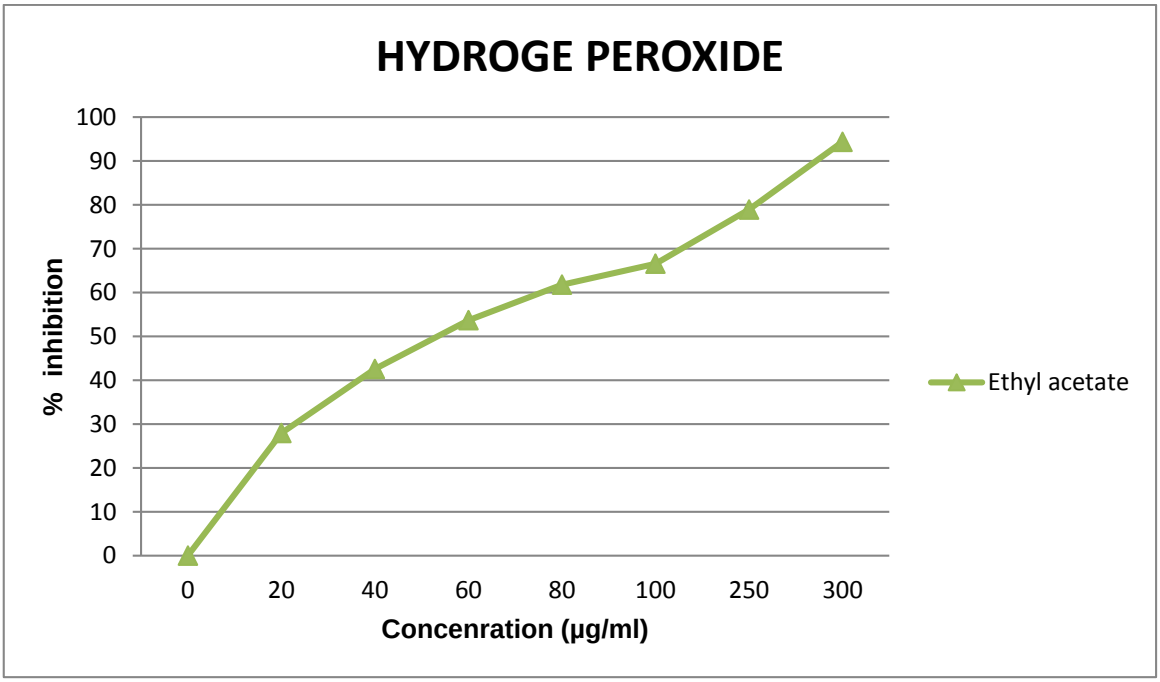
Standard-Ascorbic acid IC_{50} =5.5 µg/ml

FIGURE VII: HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF CHLOROFORM EXTRACT



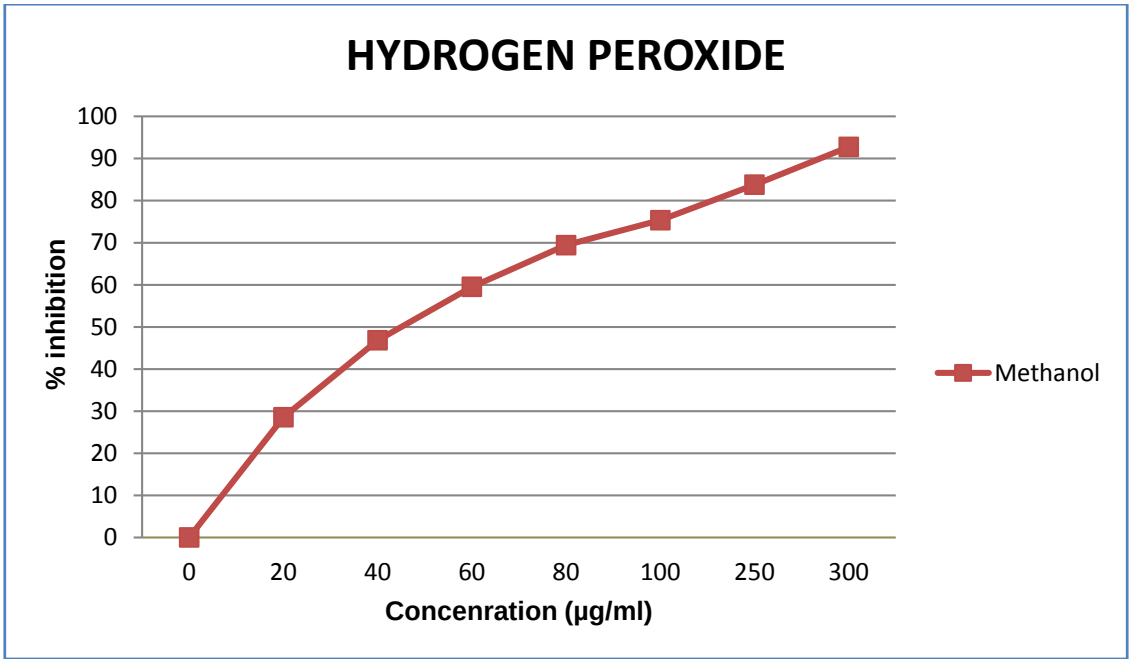
IC_{50} =31 µg/ml

FIGURE VIII: HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF ETHYL ACETATE EXTRACT



IC_{50} =46 µg/ml

FIGURE IX: HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF METHANOL EXTRACT



IC_{50} =40 µg/ml

FIGURE X : HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF VARIOUS EXTRACTS (CHLOROFORM, ETHYL ACETATE, METHANOL) AGAINST STANDARD (ASCORBIC ACID)

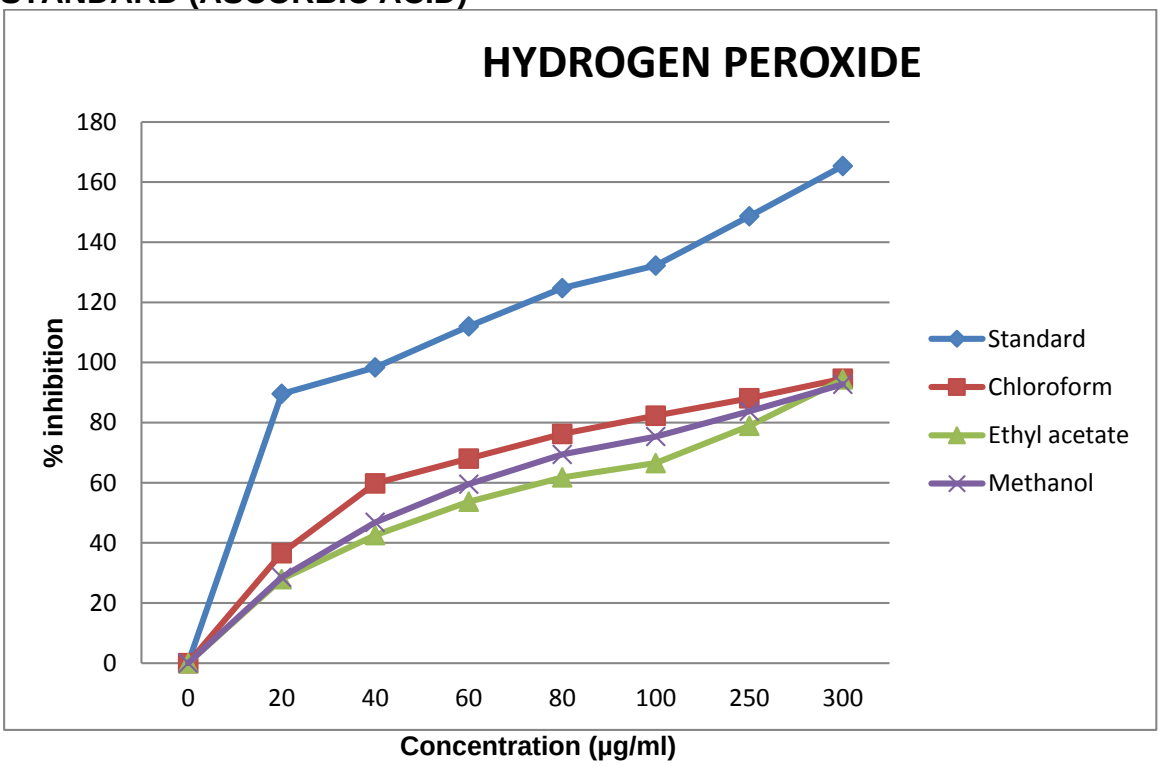
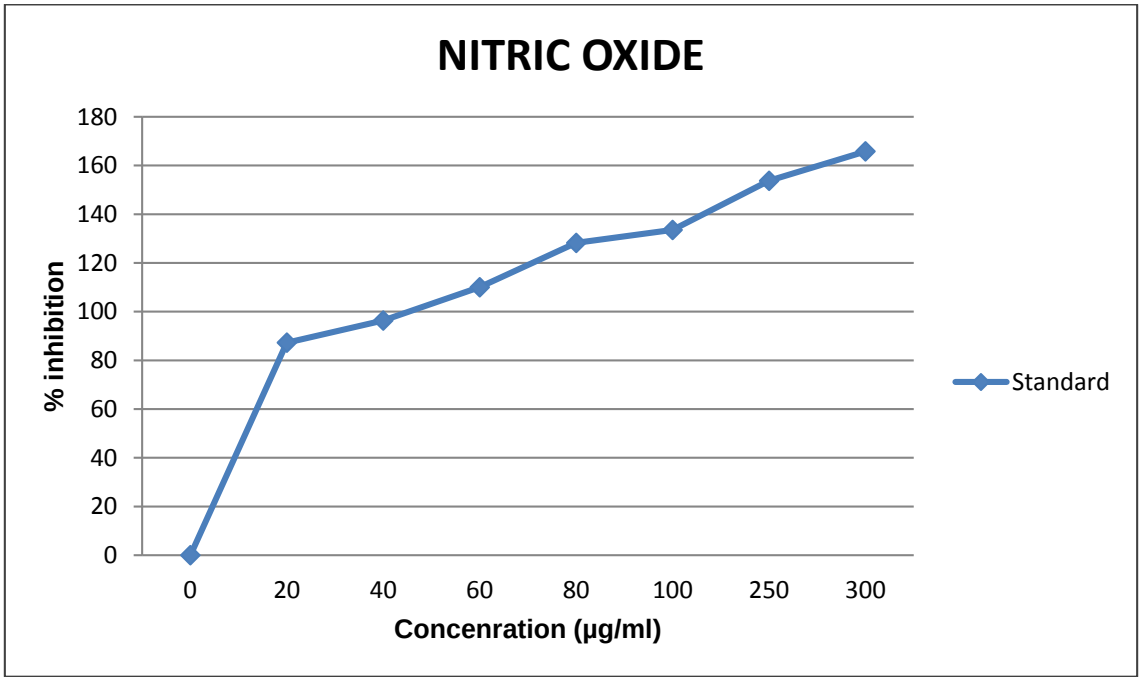
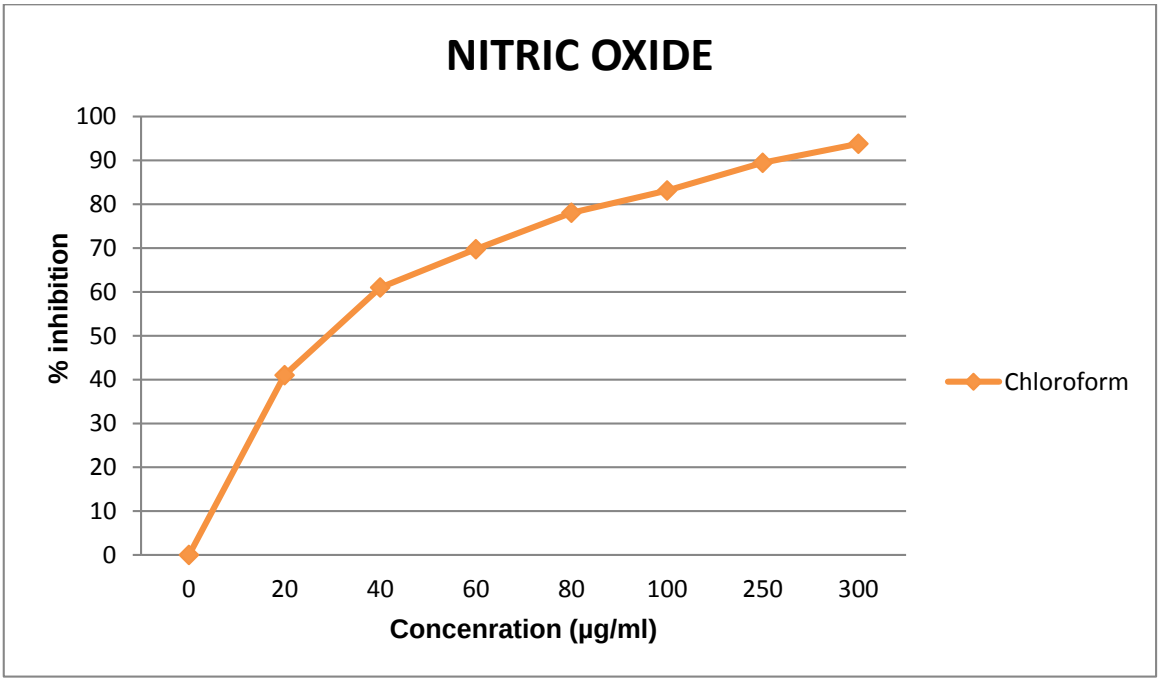


FIGURE XI: NITRIC OXIDE SCAVENGING ACTIVITY OF STANDARD (ASCORBIC ACID)



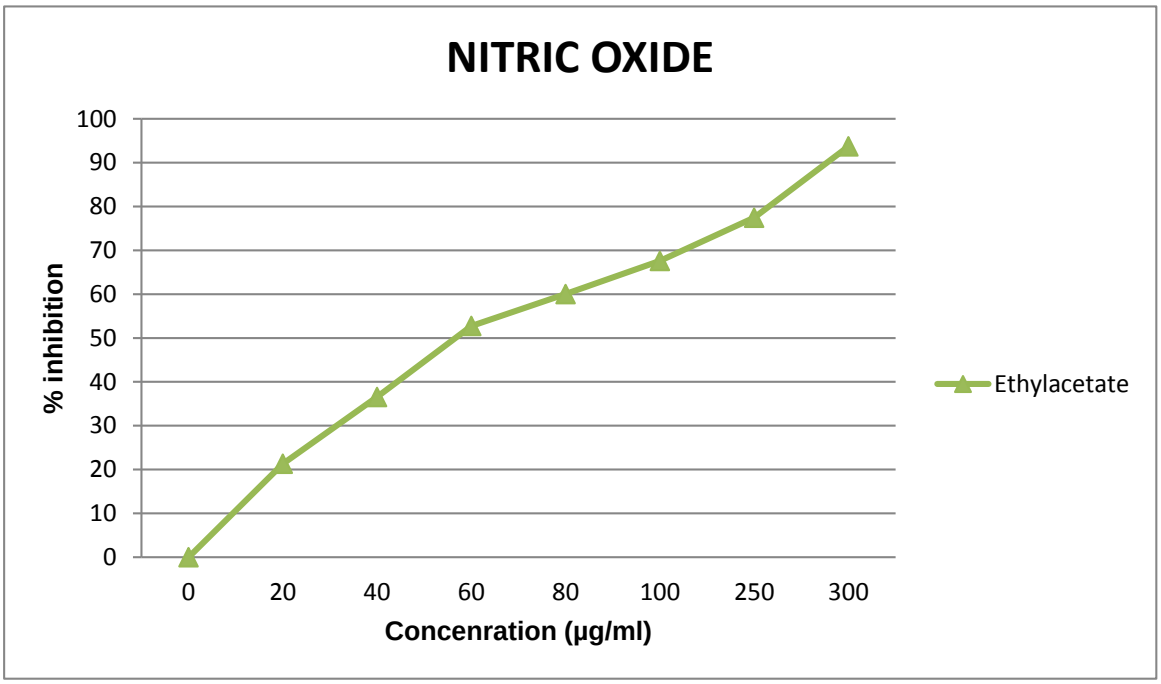
Standard-Ascorbic acid IC_{50}-6.8 µg/ml

FIGURE XII: NITRIC OXIDE SCAVENGING ACTIVITY OF CHLOROFORM EXTRACT



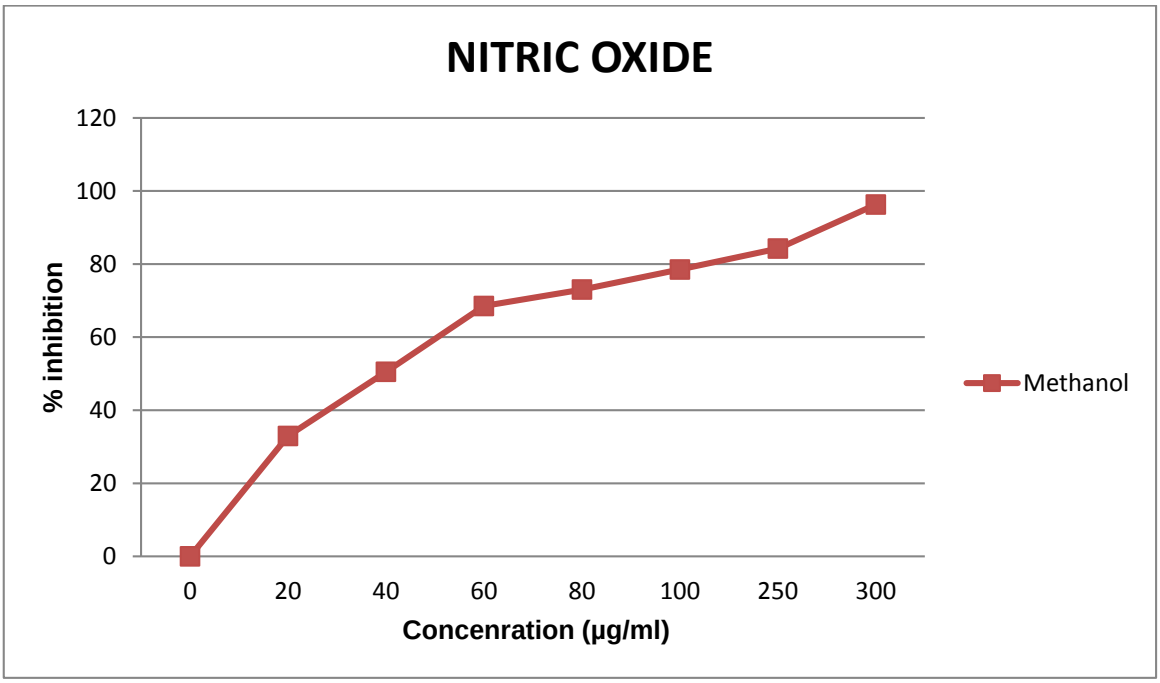
IC_{50} =39 µg/ml

FIGURE XIII: NITRIC OXIDE SCAVENGING ACTIVITY OF ETHYL ACETATE EXTRACT



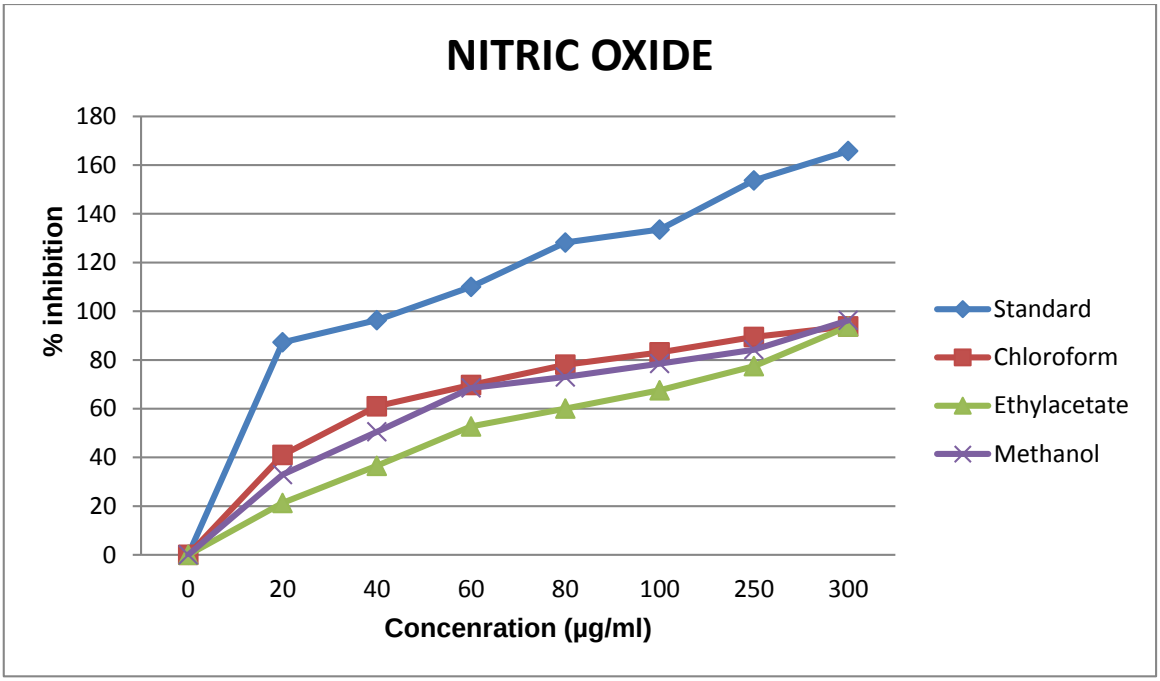
IC_{50} =46 µg/ml

FIGURE XIV: NITRIC OXIDE SCAVENGING ACTIVITY OF METHANOL EXTRACT



IC_{50} =38 µg/ml

FIGURE XV: NITRIC OXIDE SCAVENGING ACTIVITY OF VARIOUS EXTRACTS (CHLOROFORM,ETHYL ACETATE,METHANOL) AGAINST STANDARD (ASCORBIC ACID)



SUMMARY AND CONCLUSION

SUMMARY

Herbal research is still a vital source of novel pharmaceutical products moreover it is one of the best reservoirs for synthesis of novel structural based bioactive compounds. Near to 30% of the current using drugs comes from natural products and an additional 20% are structural alteration of the lead compounds from natural source. It is estimated that only 15% of higher plants have been investigated for potentially useful biological activity. In spite of the presence of known antioxidant medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease. Several traditional plant treatments like diabetes cardiac complicated disease, malaria, antiinsecticide etc. are used throughout the world. Herbal drugs and formulations are frequently considered to be less toxic and free from side effects than synthetic one. Hence, in modern days, huge attention has been directed towards recognition of plants with antidiabetic, cardiac complicated disease, malaria, antiinsecticide etc, ability that may be used effectively for human consumption. There has been rapid expansion of different classes of antioxidant drugs with distinctive pharmacological mechanism of action and, also, they have various toxicological profiles.

From the literature survey it has been found that the barks of the selected plant (*syzygium cuimini (L.)*.) are being used traditionally in the treatment of diabetes, based upon their traditional and ethnopharmacological information's

The objective of the present research work focused on the investigation of phytochemical constituents of their various extractions based on their polarity and study of *in vitro* antioxidant as well as *in vitro* antimicrobial activity.

The barks of *Syzygium Cumini (L.).* were collected from plants growing in the Krishnan koil, virudhunagar district during the months of July–August, 2016. It was then authenticated by Dr. Stephan, Dept. of Botany, The American College, Madurai.The bark was dried at 40^0 C for 15 days, then it was blended into coarse powder by electrical grinder. The powdered drug was passed through sieve No.22 to get uniform particle size. The barks (1000gm) were extracted by macerating with 2.5 litter of

Petroleum ether for 7 days at room temperature in a dark cabinet. After 7 days, the extracts were collected by filtration, the marc was separated.Solvent extract was evaporated to dryness using rotary evaporator and weighed.

The residue of petroleum ether extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.5 litter of hexane for 7 days at room temperature in a dark cabinet. After 7 days, the extracts were collected by filtration, the marc was separated. Solvent extract was evaporated to dryness using rotary evaporator and weighed. The residue of hexane extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.5 litter of chloroform for 7 days at room temperature in a dark cabinet. After 7 days, the extracts were collected by filtration, the marc was separated.Solvent extract was evaporated to dryness using rotary evaporator and weighed. The residue of chloroform extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.5 litter of ethyl acetate for 7 days at room temperature in a dark cabinet. After 7 days, the extracts were collected by filtration, the marc was separated.Solvent extract was evaporated to dryness using rotary evaporator and weighed. The residue of ethyl acetate extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.5 liter of methanol for 7 days at room temperature in a dark cabinet. After 7 days, the extracts were collected by filtration, the marc was separated.Solvent extract was evaporated to dryness using rotary evaporator and weighed. The residue of methanol extraction was dried at sun shade in dark place. After dried residue were extracted by macerating with 2.5 liter of distilled water for 7 days at room temperature in a dark cabinet. After 7 days, the extracts were collected by filtration, the marc was separated.Solvent extract was evaporated to dryness using rotary evaporator and weighed. The total ash method is designed to measure the total amount of material remaining after ignition. This includes both “Physiological ash” which is derived from the plant tissues itself and “Non physiological” which is the residue of the extraneous matter (e.g.,) sand and soil adhering to the plant surface**.**

The various extracts of *Syzygium Cumini (L.).* obtained were subjected to qualitative analysis to test the presence of various phytochemical constituents like alkaloids, carbohydrates, glycosides, flavonoids, steroids, aminoacid, phenols, proteins, tannins etc.

The obtained bark extracts of *Syzygium Cumini (L.).* were subjected to antimicrobial assay by well diffusion method and disc diffusion method, the results were posted as table III, IV. From the tables, four extracts (pet. ether, $CHCl_3$, Ethyl acetate, Methanol) of the titled plant have shows significant activity against gram + ve as well as gram - ve bacteria. Among them pet. ether extract of *Syzygium Cumini (L.).* has shows 3mm of zone of inhibitory activity about 25µg/0.1ML as shows significant activity against *B. Substilus* in well diffusion method and 3mm of zone of inhibitory activity about 25µg/0.1ML as shows significant activity against *B. Substilus* in disc diffusion method. Ciprofloxacin 10µg/0.1ML used as standard drug for both methods.

The obtained bark extracts of *Syzygium Cumini (L.).* were subjected to antioxidant assay by DPPH, H_2O_2 and NO methods, the results were posted as Fig V, X and XV. From the Fig In DPPH assay $CHCl_3$ extract of titled plant has shown as Significant scavenging activity the absorbance range from 1.342 ± 0.0016 to 0.643 ± 0.0028 (mean ± SEM) while compare with other two extracts (EA and MeOH) absorbance ranging from 1.359 ± 0.0017 (mean ± SEM) to 0.637 ± 0.0031 and 1.265 ± 0.0015 to 0.582 ± 0.0036 respectively. All the values of absorbance taken as mean of triplicates. The half inhibitory concentration (IC_{50}) of various extracts of $CHCl_3$, EA and MeOH about 41µg/ML, 48 µg/ML and 45 µg/ML respectively. In H_2O_2 scavenging activity of the $CHCl_3$ extract of the titled plant has shown significance scavenging activity, the absorbance ranging from 1.490±0.0018 to 0.623±0.0036 (mean ± SEM) while compare with other two extracts (EA and MeOH) absorbance ranging from 1.371±0.0016 (mean ± SEM) to 0.683 ±0.0033 (mean ± SEM) and 1.377 ±0.0017 (mean ± SEM) to 0.612±0.0036 (mean ± SEM) respectively. All the values of absorbance taken as mean of triplicates. The half inhibitory concentration (IC_{50}) of various extracts of $CHCl_3$, EA and MeOH about 31µg/ML, 46 µg/ML and 40 µg/ML

respectively. NO scavenging activity of the $CHCl_3$ extract of the titled plant has shown significance scavenging activity, the absorbance ranging from 1.160±0.0015 to 0.613±0.0036 (mean ± SEM) while compare with other two extracts (EA and MeOH) absorbance ranging from 1.192±0.0014 (mean ± SEM) to 0.624±0.0031 (mean ± SEM) and 1.120±0.0012 (mean ± SEM) 0.654±0.0034 (mean ± SEM) respectively. All the values of absorbance taken as mean of triplicates. The half inhibitory concentration (IC_{50}) of various extracts of $CHCl_3$, EA and MeOH about 38 µg/ML, 46 µg/ML and 39 µg/ML respectively.

CONCLUSION:

In conclusion, the present study provides evidence that barks of *Syzygium Cumini (L.).* extracts shows *significant antimicrobial as well as* potential free radical scavenging activity. These *In vitro* assays demonstrate that bark extracts of *Syzygium Cumini (L.).* are important sources of natural antioxidants, which might be useful as preventive agents against oxidative stress and hence currently the evaluation of *In vivo* antioxidant activity of these extracts are in progress.

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